The ecology of arbuscular mycorrhizal fungi in inland boreal salt pans

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Thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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ISBN 0-612-13221-8

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THE ECOLOGY OF ARBUSCULAR MYCORRHIZAL FUNGI IN INLAND BOREAL SALT PANS

BY

PERRY CLARK JOHNSON-GREEN

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Abstract

Arbuscular mycorrhizal fungi (AMF) are common inhabitants of plant roots in many terrestrial communities. The principal objective of this thesis is to assess the potential importance of AMF in ecological processes in inland salt pans of north-central Manitoba. This objective was met through a study of the distribution of AMF in plants of the salt pans, in relation to the gradient in soil salinity present at these sites. This was followed by a series of greenhouse and growth-chamber studies that examined effects of AMF on growth and competitive relationships of three halophytic grasses: *Agropyron trachycaulum* (Link) Malte; *Hordeum jubatum* L.; and *Puccinellia nuttalliana* (Schultes) Hitchc.

Arbuscular mycorrhizal fungi were common in moderately saline soil colonized by grasses such as *H. jubatum*, but were absent in highly saline soil occupied by *P. nuttalliana*. The distribution of AMF corresponded with the distribution of *H. jubatum* and *Distichlis stricta* (Torr.) Rydb., but not because of a specific relationship with these plants. Instead, they appeared to share a similar level of salinity tolerance.

Arbuscular mycorrhizal fungi suppressed the growth of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana* in greenhouse and growth-chamber experiments. This did not result in changes in the outcome of competition among these grasses, despite a disparity between the amount of suppression suffered by *P. nuttalliana* and the other two species.

In the course of this project, it became evident that there is substantial potential for within-site and between-site variation in the effects of AMF on their hosts. This variation appears to be caused by variation in the number of AMF-propagules in the soil, and variation in the presence and density of pathogenic and growth-stimulatory non-mycorrhizal microorganisms.

In conclusion, AMF were common inhabitants of roots of a variety of plant species in inland salt pans, indicating that AMF possess considerable salt tolerance. They are capable of strongly affecting the growth of halophytic grasses, and may well be important in ecological processes in saline habitats.

Acknowledgements

My advisors, Dr. Norm Kenkel and Dr. Tom Booth, provided indispensable advice, guidance, and support during my years in Winnipeg, as well as timely assistance in the field and in the lab. The other members of my advisory committee, Dr. William Woodbury and Dr. Mike Sumner were a source of many great ideas and useful discussions. The faculty and staff of the Botany Department of the University of Manitoba have been very generous in their help, support and encouragement; I would particularly like to thank Margaret Smith, Mark Elliot, Dr. J. Reid, and Dr. D. Punter. Dr. R. Hunt of the Freshwater Institute generously provided instruction and the use of equipment. Charles Burchill and Geoff Jones wrote excellent theses that I found extremely useful. I would also like to thank my friends in the lab next door (Jason Greenall and Barbara Dyck) and upstairs (Eun Ju Lee) for many enjoyable discussions.

I have obtained monetary support from the Faculty of Graduate Studies, and the Northern Studies Committee of the University of Manitoba, for which I am grateful. Much of the research described in this thesis was also funded by an NSERC Research Grant received by Dr. Norm Kenkel.

The field and lab work required for this thesis was aided immeasurably by the efforts (in chronological order) of Geoff Jones, Mike Shaw, Julia Green-Johnson, Katherine Green, Krista Copeland, and Kaisa Junninen. I would especially like to thank Krista and Norm for lugging many heavy plants all over northern Manitoba.

Finally, I would like to thank Julia Green-Johnson, Hugh Johnson, Suzanne Johnson, and Katherine Green for their boundless encouragement and support during the last four years.

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List of Abbreviations

AMF	arbuscular mycorrhizal fungi.
ANOVA	analysis of variance.
CI	colonization intensity.
EMF	ectomycorrhizal fungi.
ERMF	ericoid mycorrhizal fungi.
YS	yield suppression.
MPN	most probably number.
PRA	proportion of root segments with arbuscules.
PRC	proportion of root segments with AMF-colonization.
PRLA	proportion of root length with arbuscules.
PRLC	proportion of root length colonized by AMF.
PRLV	proportion of root length with vesicles.
PRV	proportion of root segments with vesicles.
RSR	root to shoot ratio.
SB	shoot biomass.
TCRL	total root length colonized by AMF.
TRL	total root length.

Chapter 1

General Introduction

A. Introduction to mycorrhizae

Mycorrhizae are mutualistic associations between fungi and plant roots. Colonization of roots by mycorrhizal fungi is extremely common in terrestrial plant communities; indeed, uncolonized root systems are rare in many habitats (Mosse, 1981). Mycorrhizae have been divided into four types based on the taxonomic affinity of the fungus, and the structure of the mycorrhiza. They can also be separated on the basis of the taxanomic affinity of the host, although in some cases (*e.g. Populus tremuloides*) more than one type of mycorrhiza can be associated with a plant species.

Orchid mycorrhizae are characterized by intracellular coils of hyphae of *Rhizoctonia* in orchid roots. A different group of fungi (*e.g. Hymenoscyphus* -an ascomycete) forms ericoid mycorrhizae, which are also characterized by intracellular coils in roots; in this case roots of ericaceous plants are colonized. Conifers and many trees and woody shrubs are colonized by ectomycorrhizal fungi (EMF), which can be Ascomycetes or Basidiomycetes, and typically form a dense "mantle" of hyphae surrounding root tips. Most tropical trees and temperate herbs, forbs and grasses form associations with arbuscular mycorrhizal fungi (AMF), which are Zygomycetes that colonize the cortex of roots. Since AMF are the focus of this thesis, the other types of mycorrhizae will not be discussed further.

There are several aspects of AMF-biology that have ecological ramifications. Firstly, virtually all AMF species (<200 in total) belong to four genera: *Glomus*, *Gigaspora*, *Acaulospora*, and *Scutellospora*. Since these four genera are capable of

colonizing 50% of the 6,507 angiosperm species that have been studied (Trappe, 1987), host-specificity of AMF must be very low. Indeed, most AMF-isolates are capable of colonizing plants from a wide variety of families, orders, and classes (Molina *et al.* 1992). Plant response to AMF, however, varies among AMF-species, leading to particular host-fungus combinations that are favorable, or unfavorable to plant growth (Johnson *et al* 1992).

Although a large number of vascular plants are amenable to AMF-colonization, there are also families of plants that are normally free of mycorrhizal colonization (Trappe, 1987, Tester *et al.* 1987). The primary mechanism of AMF-resistance in these plants may be the inhibition of the initial penetration of the epidermal cell layer (Tommerup, 1984, Tester *et al.*, 1987).

Non-mycotrophic plants (plants that do not normally form mycorrhizae in the field) and mycotrophic plants (plants that normally form mycorrhizae in the field) often coexist in plant communities. The amount of AMF present in a particular community can have important effects on interactions between mycotrophic and non-mycotrophic species, that in turn affect ecological processes such as plant competition and succession (Allen and Allen, 1984, 1988).

The relative importance of AMF in plant community function can be expected to vary with edaphic factors such as availability of phosphorus (Sparling and Tinker, 1978*a*), organic matter (Menge *et al.*, 1981), and soil moisture (Anderson and Liberta, 1986). The importance of AMF is also thought to decrease as climates become cooler (Koske, 1987). Thus, the effects of AMF on ecological processes should not be regarded as a constant factor, but one that varies depending on the plant and fungal species present, local edaphic factors, and regional climatic factors.

B. Life-cycle of arbuscular mycorrhizae

Arbuscular mycorrhizal fungi, unlike most fungi, are unable to grow in pure culture, and cannot complete their life cycle in the absence of plant roots. Consequently, an essential part of the AMF life-cycle is the process of colonization of roots. Colonization occurs through the growth of hyphae from propagules such as spores, colonized roots, or dormant hyphae (Bowen, 1987). Growth of hyphae growing from germinating spores is stimulated by root exudates (Elias and Safir, 1987) and volatile compounds produced by roots (Koske, 1982; Gemma and Koske, 1988). Arbuscular mycorrhizal fungal hyphae branch profusely when they are close to the root (Becard and Piche, 1989), which presumably increases the probability that a branch will contact the root.

When a hypha encounters a root, the hyphal tip swells, and an appressorium forms, followed by entry of the hypha into the root. The hypha typically grows between the cell walls of adjoining epidermal cells (Bowen, 1987). The fungus then grows intercellularly through the cortex of the root, and eventually forms intracellular structures, usually in the inner cortex of the root. Either vesicles or arbuscules are produced. Vesicles are enlarged hyphae that are lipid-rich and appear to be dormant structures (Bowen, 1987).

Arbuscules are distinct structures that are formed by repeated dichotomous branching of the hyphal tip. This produces an enormous hyphal surface area, and nutrient exchange between host and fungus occurs at the interface between the plasmalemma of the arbuscule, and the plasmalemma of the cortical cell (Bonfante-Fasolo and Scannerini, 1992). Arbuscules are relatively short lived (4-15 days -Cox and Tinker, 1976), and are a useful indicator of mycorrhizal activity.

Hyphal growth is not limited to the interior of the root; arbuscular mycorrhizal hyphae also grow extensively in the soil (Read, 1992). These soil-hyphae have two

functions: they colonize new roots (of the same plant, or of other plant individuals or species); and they absorb phosphate from the soil, translocate it to the arbuscule, and transfer it to the plant cell that contains the arbuscule (Bonfante-Fasolo and Scannerini, 1982). Hyphae growing in the soil also produce spores, which, along with soil hyphae and colonized roots, are mechanisms of dormancy that allow AMF to survive unfavorable environmental conditions.

C. The nature of host benefit from AMF

AMF benefit their host plants through improved mineral nutrition, primarily of poorly mobile nutrients such as phosphorus (P), zinc (Zn), and copper (Cu)(Hayman, 1983). Fungal hyphae in the soil take up these nutrients, translocate them to plant roots, and transfer them to the host via arbuscules.

The principal advantage of fungal uptake, as compared with root uptake of poorly mobile nutrients, is the ability of AMF-hyphae to grow beyond depletion zones of P (Hayman, 1983) at relatively low cost to the root. Other mechanisms of mycorrhizal benefit include improvements in host drought tolerance and disease resistance. These are often side-effects of improved P-nutrition (Nelsen and Safir, 1982, Smith, 1988) but direct benefits arising from mycorrhizal colonization have also been documented (Augé *et al* 1986, Garcìa-Garrido and Ocampo, 1988). In these cases the beneficial action of AMF is probably related to improved uptake of water through mycorrhizal hyphae (Augé *et al* 1986), or to stimulation of the production of anti-fungal metabolites (Garcìa-Garrido and Ocampo, 1988).

Mycorrhizae are usually considered to be mutualistic associations, but host plants often do not benefit, and may suffer from AMF-colonization (Miller, 1987). Growth depression in AMF-colonized plants may be caused by adverse environmental conditions

such as low soil temperature (Furlan and Fortin, 1973), that inhibit normal function of the symbiosis. Alternatively, in plants such as *Lolium perenne,* pathogenicity may be a normal facet of the mycorrhizal relationship (Buwalda and Goh, 1982). *L. perenne* has well-developed root systems, with frequent branches and abundant root hairs. Mycorrhizal colonization confers little benefit to plants with extensive root systems, since they are already capable of efficiently extracting phosphorus from soil (Baylis, 1975, Miller, 1987). The host plant still transfers carbon to the fungus, though, which may lead to growth depression of the plant.

D. Host benefit in the field

Demonstrations of mycorrhizal benefit to host plants has usually involved the comparison of mycorrhizal and non-mycorrhizal plants grown in a greenhouse or growth chamber. Attempts to measure the extent of host benefit in the field have met with variable results, that are often difficult to interpret (Fitter, 1985). Field-grown mycorrhizal plants are often similar in growth and nutrition to non-mycorrhizal plants (*e.g.* Sparling and Tinker, 1978*b*). The difficulty in clearly demonstrating mycorrhizal benefit in the field, as compared to in the greenhouse, may arise from the presence in field soils of a diversity of organisms that graze on mycorrhizal hyphae (Fitter, 1985). Soil-borne bacteria and fungi are also capable of decreasing host-benefit from AMF (Hetrick *et al* 1988). Another complicating factor in many soils is the presence of indigenous AMF-species that do not stimulate plant growth (Powell, 1979, Rangely *et al.* 1982). This may be caused by a diversion of AMF-resources to spore production rather than to hyphal growth and nutrient uptake (Johnson *et al.* 1992).

E. Objectives

The objective of this thesis was to study the ecology of mycorrhizal fungi in inland salt pans in north-central Manitoba. As mentioned previously, edaphic factors can have important effects on mycorrhizal ecology. In contrast to the wealth of information on the relationship between soil nutrients and mycorrhizae, little is known of the impact of soil salinity on mycorrhizal fungi. My overall aim was to explore 3 facets of mycorrhizal ecology in inland salt pans: (*i*) the spatial and temporal distribution of AMF-colonization in plants throughout a salt pan (Chapter II); (*ii*) the nature of the response of grasses common in the salt pans to inoculation with AMF under greenhouse and growth-chamber conditions (Chapter IV); (*iii*) effects of AMF on interspecific competition between these grasses (Chapter VI).

Several other aspects of AMF-ecology are also addressed. Chapter III describes a study of the correspondence between vegetation zones and mycorrhizal zones in a salt pan, and Chapter VII contains results of a preliminary study of mycorrhizal colonization of plants in saline and non-saline habitats in a transition zone (Churchill, Man.) between the boreal forest and the low arctic. Chapter V addresses a problem that is seldom discussed: in many communities there may be substantial local variation in plant response to AMF; this variation can have many sources, and it may have important implications for the function of AMF in natural systems. It also has implications for the design of experiments that aim to increase understanding of the importance of AMF in natural systems.

The results of this thesis are important not only in the context of natural saline systems, but also in the context of saline agricultural land. The ability of AMF to survive in saline soil has important implications for their potential use in improving crop or forage growth in saline soil. AMF may also be useful in the restoration of severely disturbed coastal salt marshes (Cooke and Lefor, 1990).

Chapter II

The Distribution and Phenology of Arbuscular-Mycorrhizae along a Salinity Gradient at an Inland Salt Pan

A. Abstract

The distribution and seasonal patterns of activity of arbuscular mycorrhizal fungi (AMF) were studied in an inland boreal salt pan in north-central Manitoba. Semi-permanent plots were set up in each of five vegetation zones along a continuous salinity gradient. Root samples of *Hordeum jubatum*, *Distichlis stricta*, *Agropyron trachycaulum*, *Sonchus arvensis*, and *Spartina gracilis* were collected from the plots at six periods: April, June, July, August, and Ocober of 1991, and May of 1992. These root samples were used to quantify AMF-colonization, as well as arbuscule and vesicle formation.

AMF were prevalent in the three vegetation zones with lowest soil salinity (greater than 40% of the root pieces observed were colonized). There was less than 2% colonization in the 2 other zones, where soil salinity was consistently greater throughout the growing season.

The only common pattern in the phenology of AMF-activity was a low level of activity in the early spring. Activity of AMF in most plant species occurred at a high level throughout the summer and fall. Differences in patterns of activity appeared to be linked to differences in phenology of root growth, and not to environmental differences among vegetation zones.

B. Introduction

AMF are commonly found in a diversity of plant communities throughout the world (Mosse et al. 1981). Although many greenhouse studies have demonstrated benefit to the plant host from the association, it has been difficult to demonstrate benefit to plants in the field (Fitter 1991). This has created problems in assessing the importance of mycorrhizal fungi in natural communities. One approach taken to address this has been to collect roots from a range of species (e.g. Brundrett and Kendrick 1988). Mycorrhizae are potentially important if mycorrhizal colonization is frequently observed within a community.

Tolerance to adverse environmental conditions is likely to be an important factor determining the importance of mycorrhizae in many communities. The study of gradients of edaphic factors can provide information on the extent of an organism's tolerance, because different levels of a factor can be examined at the same site, without confounding effects of site-to-site differences.

Gradients in soil salinity are common in inland and coastal systems, but have rarely been the focus of studies on mycorrhizal fungi. Soil salinity exerts strong effects on individual plant species and on plant community structure (Ungar 1991). It would also be expected to strongly affect mycorrhizal fungi. Mason (1928) and Sengupta and Chaudhuri (1990) observed AMF in several species of salt marsh plants. Rozema et al. (1986) found that the distribution of AMF in a coastal salt marsh in the Netherlands was related to taxonomic affinities of plants (mycotrophic vs non-mycotrophic families) rather than to spatial position in the marsh. Spatial position would be expected to be important in coastal marshes, because of the effects of tidal inundation on soil salinity and on soil aeration.

Inland salt pans offer the opportunity to study the effects of soil salinity in the absence of the confounding influence of tidal inundation. Two studies of North American

inland saline communities have documented a negative relationship between mycorrhizal abundance and soil salinity (Kim and Weber 1985; Ho 1987). However, in these studies mycorrhizal presence was not differentiated from AMF-activity. The observation of arbuscules within roots indicates that AMF are active within roots. In contrast, hyphae or vesicles within roots may be part of actively growing, dormant, or dead mycorrhizae. Furthermore, the potential importance of seasonal changes in mycorrhizal abundance was not considered. The goal of this chapter is to examine the distribution of AMF-activity at an inland boreal salt pan throughout the growing season. This will also provide information on the relationship between mycorrhizal phenology and host phenology, which may be important to the understanding of the role of AMF in natural systems (Sanders and Fitter 1992a). If AMF are important to their hosts, then peak times of host growth should be accompanied by high levels of AMF-activity.

One potential problem associated with this idea is that seasonal patterns in AMF-activity could be linked to seasonal changes in the environment, and may be unrelated to patterns of host activity. The comparison of different host species helps differentiate between these two possibilities. If phenological patterns of AMF-activity vary with different host species within a site, then the fungi are responding to seasonal changes in host growth. However, if the phenology of AMF-activity is constant across host species, then the fungi are probably reacting directly to seasonal environmental signals.

The objectives of this study, then, were (*i*) to determine the distribution of AMF at various points along a salinity gradient at an inland boreal salt pan, and (*ii*) to compare the phenology of AMF-activity in five grass species at this site.

C. Study area

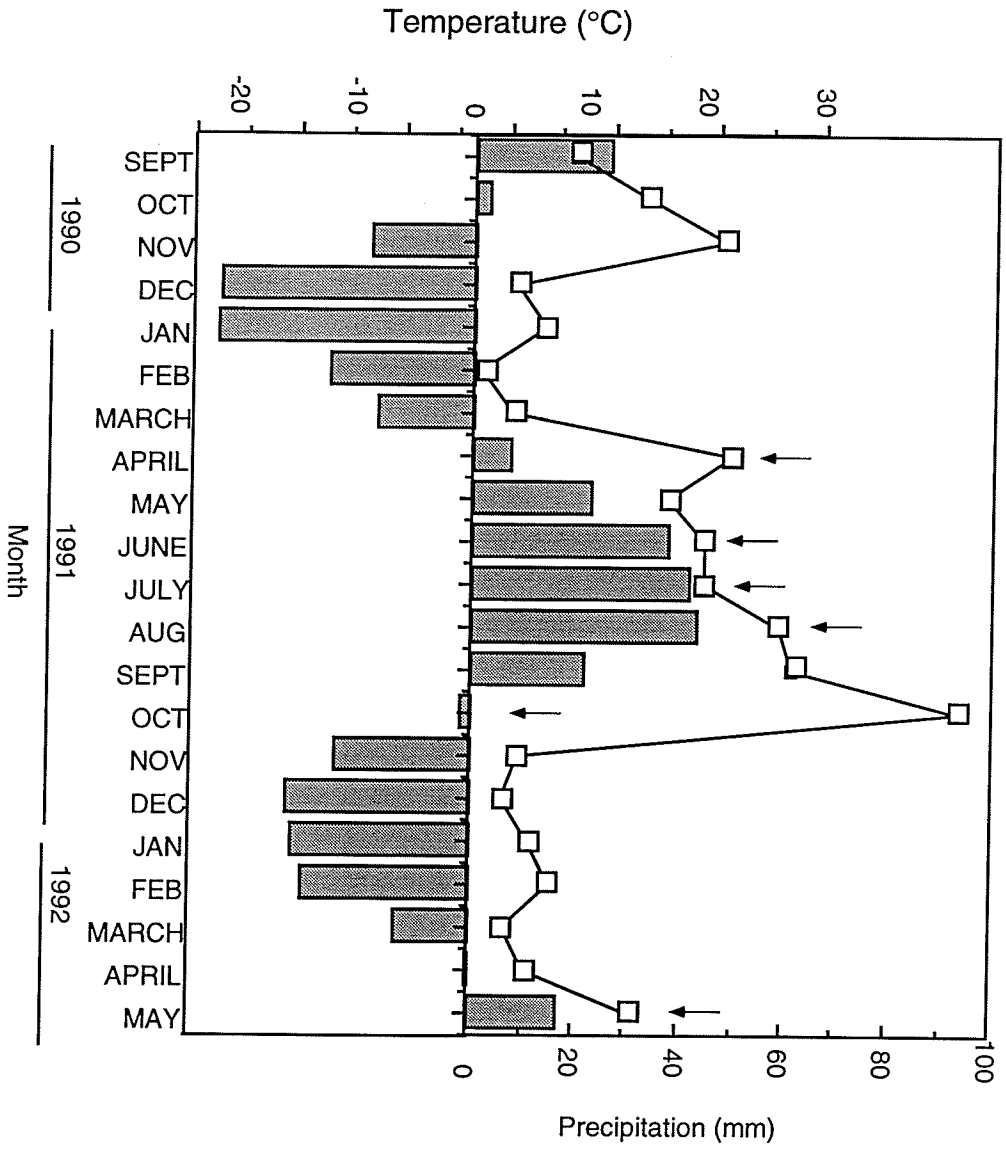
The study area is described in Burchill and Kenkel (1991). The site is located approximately 10 km south of the town of Overflowing River, and 1 km from the shore of Overflow Bay, Lake Winnipegosis, Manitoba, Canada (53°05'N and 101°07'W). It is ≈600 km northwest of Winnipeg. Salt pans in this region occur when subterranean saline water flows to the surface. The dominant ions in saline springs in this area are Na^+ and Cl^- (Everingden 1971; McKillop et al. 1992).

Concentric zones of vegetation occur around an unvegetated salt pan. *Salicornia rubra* A. Nels. is found on margins of these salt pans, and zones characterized by the grasses *Puccinellia nuttalliana*, *Hordeum jubatum*, *Agropyron trachycaulum*, and the shrub *Rosa acicularis* Lindl. occur in progressively more elevated, and less saline soil. Nomenclature follows Looman and Best (1987).

The soil is a rego gleysol of the series 'saline flats' (Hopkins and Smith 1982). The parent material is made up of recently exposed lake flats, and is very calcareous. The pH is somewhat alkaline (7-8), and the level of organic matter is typically lower (5-15%) in the Salicornia zone than in the less saline zones (20-80%)(Jones 1991).

The climate is typical of the boreal forest, with a long, cold winter and a short, cool summer (Figure 1). Precipitation occurs throughout the year. Increased temperatures in July and August lead to increased evapotranspiration, and high soil salinity, especially in August (Jones 1991).

Figure 1. Monthly mean temperature (*closed squares*) and total precipitation (*open squares*) at The Pas, Manitoba, for the period: Sept., 1990 - May, 1992 (data obtained from Environment Canada). The Pas is 100 km N of the Overflowing River salt pan. Arrows indicate months when sampling took place.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JAN
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MARCH
APRIL
MAY
1990
1991
1992
Month
0
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80
100
Precipitation (mm)

D. Materials and Methods

1. Field sampling

A semi-permanent 5 x 5 m plot was set up in each of the five vegetation zones (Table 1). Five randomly-selected root systems of 2 species were excavated from each plot during each of 6 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). Only 1 species (*S. rubra*) was collected from the Salicornia zone. 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 *S. arvensis* (Agropyron zone), and *R. acicularis* (Rosa zone) were collected when live roots of these species were present. During times of sparse root growth other species were sampled. A total of 10,600 root pieces from 212 root systems were examined.

2. 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 -see also Appendix A). 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 250x 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 a reliable indicator 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.

Table 1. Characteristics of vegetation zones in the salt pan.

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

[a]mean ± SD of five samples from each zone in August, 1991
[b]mean ± SD (from Burchill and Kenkel, 1991)
[c]sample size

The advantage of this method over the often-used gridline-intersect procedure is that it allows the inspection of roots at high magnification, which reduces the subjectivity of the identification of mycorrhizal structures (McGonigle et al. 1990). It also allows the quantification of arbuscules and vesicles. However, the root-piece method has been criticized because it consistently over-estimates 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 cm to 0.5 cm, and root segments from the July, August, October, and May collections were scored on a 0-4 scale for the intensity of colonization. Scores of 0,1,2,3 and 4 respectively refer to 0, 1-25, 26-50, 51-75, and 76-100% colonization of the root cortex. Such estimates are not possible with any of the 'intersection' methods (McGonigle et al. 1990).

3. Soil analysis

Soil samples from the top 10 cm of soil were taken concurrently with root samples. Soil salinity was estimated from dilution-extracts, not saturation pastes, in order to reduce the amount of soil required, thus minimizing disturbance of the site. Ten g of dried soil were mixed with 50 g deionized water and shaken for 1 h. Extracts were passed through filter paper, and pH and electrical conductivity (EC) of the extracts were measured, using a platinum electrode and an Analytical Instrument Science (Houston, USA) conductivity meter. Total salt content of the extract (640 x EC in $dS.m^{-1}$ -Rhoades and Miyamoto 1990) and the gravimetrically determined soil moisture content were used to calculate total salts (mg) per mL of soil water.

Additional soil samples were taken from the Agropyron, Hordeum, and Puccinellia zones in August, 1992, to measure extractable calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), nitrate, and phosphate in the soil. Norwest Laboratories

(Winnipeg, Canada) performed the analyses (nitrate and phosphate were measured colorimetrically, and other elements by ICP spectrophotometry).

4. Estimation of the number of AMF-propagules in the salt pan

The number of AMF-propagules in soil from each vegetation zone was estimated by a soil-dilution bioassay. The initial dilution of soil samples was 1/10, followed by 5 serial dilutions of 1/3 (only 3 dilutions for soil from the Salicornia and Puccinellia zones). Each dilution was replicated 6 times, and the diluent was autoclaved soil (121°C for 1 h) from the same zone as the diluted, non-sterile soil. To allow for potential differences in mycorrhizal host-specificity (Adelman and Morton, 1986), each dilution-series was repeated to allow the use of two "bait" plants: *A. trachycaulum*, and an additional species that was common in each zone. Bait plants were seeded into 5 cm pots containing diluted soil, and grown in a greenhouse with 130 $\mu E.m^{-2}.sec^{-1}$ supplemental illumination. Each pot was thinned to 5 plants, and after 85 days, the roots from each pot were stained (Appendix A). Each of 192 samples was scored for AMF-colonization using a stereomicroscope. Most-probable numbers (MPN) of AMF-propagules were calculated for each zone (Alexander, 1965).

5. Data analysis

To reveal seasonal trends in AMF-activity, I analyzed colonization data of *Spartina gracilis* Trin., *A. trachycaulum*, *Sonchus arvensis* L., *H. jubatum*, and *D. stricta*. Colonization of each species was analyzed separately. The proportion of roots with AMF-colonization (PRC), proportion of roots with arbuscules (PRA), and proportion of

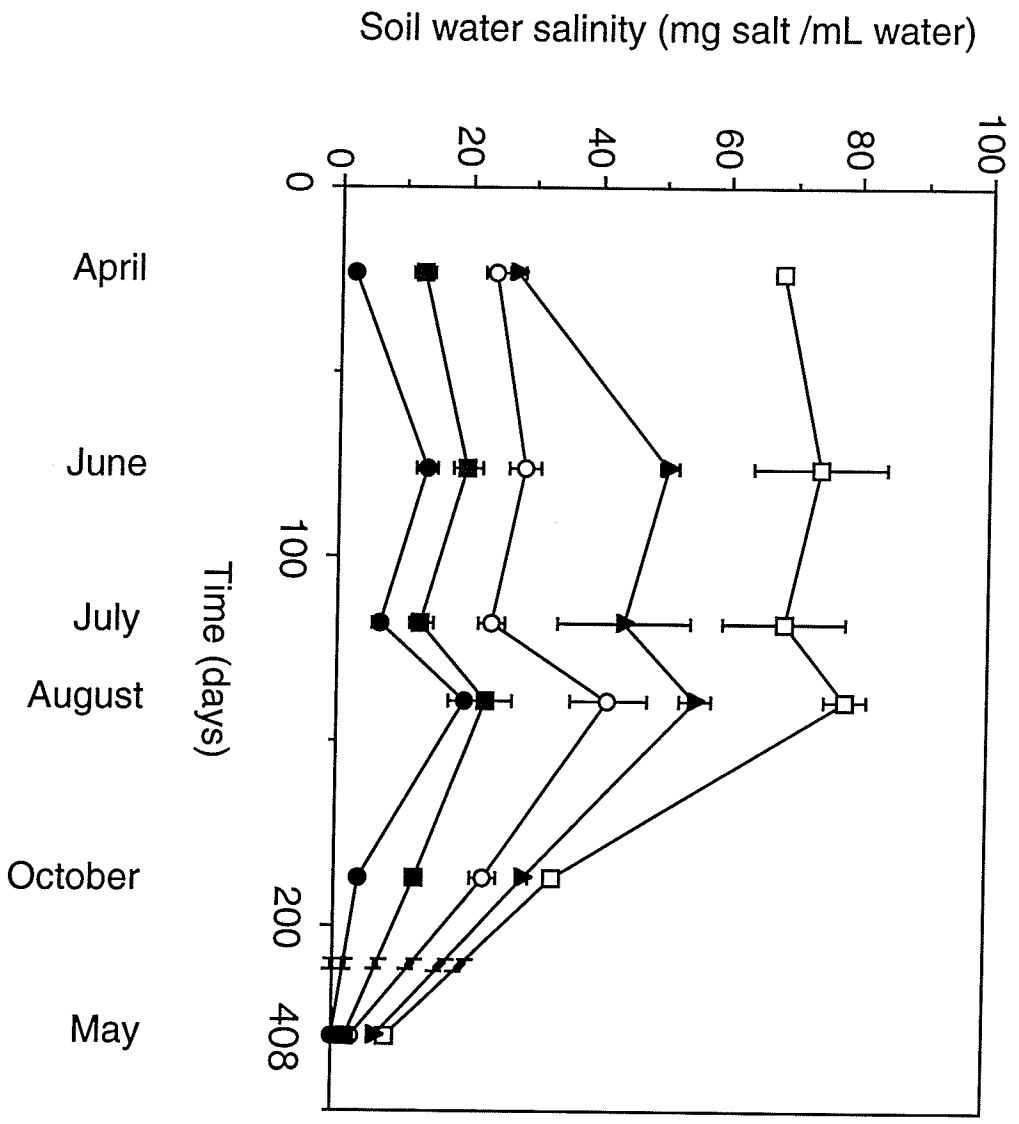
roots with vesicles (PRV) were each analyzed by one-way ANOVA to test the significance of differences among sampling times. The significance of differences between individual sampling times was tested by examining for overlap of 95% confidence intervals. In order to conform to assumptions of ANOVA (normal distribution of residuals) variables that were proportions were arcsin square root transformed before analysis (Ott, 1988). Colonization intensity was analyzed in a similar manner, although without transformation, since residuals were normally distributed. When transformation did not succeed in fulfilling ANOVA assumptions, results of ANOVA were compared to results of a non-parametric Kruskal-Wallis test. Unless otherwise stated, differences were considered statistically significant if $p<0.05$. Soil salinity was analyzed by two-way ANOVA, with the fixed factors of sampling time and vegetation zone. Soil nutrient levels were analyzed by one-way ANOVA.

E. Results

1. Soil analysis

At each sampling time except May, 1992, I found a consistent hierarchy of soil salinity; it was greatest in the Salicornia zone, and progressively decreased in the Puccinellia, Hordeum, Agropyron, and Rosa zones (Figure 2). The statistical analysis of soil salinity must be interpreted with caution, because of extreme differences in variance among sampling times. Two-way ANOVA revealed significant differences ($p<0.001$) in both factors (sampling time and vegetation zone), and a significant interaction ($p<0.001$) between sampling time and vegetation zone. Soil salinity was significantly greater in August than at all other times except June. Soil salinity was very low in May. In both

Figure 2. Soil water salinity in vegetation zones of the salt pan. Each symbol is the mean ± SE of five values. Day 0 = April 1, 1991. Salicornia zone (*open square*); Puccinellia zone (*closed triangle*); Hordeum zone (*open circle*); Agropyron zone (*closed square*); Rosa zone (*closed circle*).


Soil water salinity (mg salt /mL water)
0
20
40
60
80
100
April
June
July
August
October
May
Time (days)
0
100
200
408

1992 and 1993 soil was saturated in the Salicornia, Puccinellia and Hordeum zones during May, perhaps indicating that this is the time of peak groundwater seepage.

Nutrient analysis of soils sampled in August, 1992 revealed that soil from the Puccinellia zone had much higher concentrations of extractable Ca than did the Hordeum or Agropyron zones (Table 2). In contrast, Na levels (and levels of other nutrients) were not significantly higher in the Puccinellia zone. It therefore appears that the increase in salinity observed in the Puccinellia zone may be predominantly caused by Ca, rather than by Na.

2. 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). Mean PRC was less than 2% in each plant species from the Salicornia and Puccinellia zones, and greater than 40% in each plant species from the other zones. This difference was also apparent when uncolonized root systems were excluded; mean PRC of colonized root systems in the Hordeum, Agropyron, and Rosa zones was 76.0 ± 2.0, and in the Puccinellia and Salicornia zones it was 4.5 ± 1.0. Therefore, AMF-colonization was rare in plants growing in highly saline soil, and, when it occurred, fewer roots were colonized than in plants in less saline soil.

In plants from the Hordeum, Agropyron, and Rosa zones, arbuscules were more often observed (PRA: 47.4% ± 2.5) than vesicles (PRV: 21.6% ± 2.0). *R. acicularis*, *Aster pauciflorus* Nutt., *Aster pansus* (Blake) Cronquist and *Calamagrostis inexpansa* A. Gray were exceptions, having colonizations dominated by vesicles (*C. inexpansa* and *A. pauciflora*, however, were only sampled during April, 1991, and May, 1992, times of

Table 2. Soil nutrients in three vegetation zones at an Overflowing River salt pan.

Vegetation zone	N[a] (mg/g)	P[b] (mg/g)	K[b] (mg/g)	Ca[c] (mg/g)	Mg[c] (mg/g)	Na[c] (mg/g)
Agropyron	56 ± 24	20 ± 9	402 ± 198	10854 ± 6129	677 ± 82	7227 ± 2119
Hordeum	83 ± 3	11 ± 5	508 ± 92	10093 ± 5545	714 ± 67	10138 ± 2393
Puccinellia	43 ± 7	13 ±1	>600	71450 ± 9958	891 ± 11	12408 ± 238

[a]Calcium chloride extraction

[b]Acetic fluoride extraction

[c]Ammonium acetate extraction

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

Host species	Host family	Vegetation zone	Roots with AMF[a]	Roots with arbuscules[b]	Roots with vesicles[c]	n[d]	Month of sampling[e]
Salicornia rubra A. Nels.	Chenopodiaceae	Salicornia	0.9 ± 0.7	0.1 ± 0.1	0.6 ± 0.6	16	Jn,Jl,Au,Oc
Suaeda depressa (Pursh) S. Wats.	Chenopodiaceae	Puccinellia	0	0	0	7	Jl, Au
Puccinellia nuttalliana (Schultes) Hitchc.	Poaceae	Puccinellia	1.5 ± 2	0.7 ±0.3	0.8 ± 0.3	28	Ap,Jn,Jl,Au,Oc,Ma
Triglochin maritima L.	Triglochinaceae	Puccinellia	1.4 ± 0.9	0.3 ± 0.1	0.9 ± 0.6	23	Ap,Jl,Au,Oc,Ma
Hordeum jubatum L.	Poaceae	Hordeum	74.3 ± 25.0	58.9 ± 5.6	7.4 ± 1.0	24	Ap,Jn,Jl,Au,Oc,Ma
Aster pauciflorus Nutt.	Asteraceae	Hordeum	50.5 ± 9.6	8.3 ± 3.7	29.4 ± 6.6	2	Ma
Distichlis stricta (Torr.) Rydb.	Poaceae	Hordeum	75.7 ± 3.1	57.6 ± 5.0	10.2 ± 1.9	15	Jn,Jl,Au,Oc
Atriplex patula L.	Chenopodiaceae	Hordeum	70.0	42.0	28.0	1	Au
Grindelia squarrosa (Pursh) Dunal	Asteraceae	Hordeum	87.0 ± 9.0	81.0 ± 9.0	9.0 ± 1.0	2	Jl
A. trachycaulum (Link) Malte	Poaceae	Agropyron	79.4 ± 3.3	58.4 ± 4.0	15.9 ± 1.2	29	Ap,Jn,Jl,Au,Oc,Ma
Aster pansus (Blake) Cronquist	Asteraceae	Agropyron	50.2 ± 18.6	15.9 ± 9.9	22.9 ± 13.9	6	Ap, Ma

Sonchus arvensis L.	Asteraceae	Agropyron	90.1 ± 3.5	15.9 ± 9.9	26.0 ± 4.0	6	Jl, Au,Oc,Ma
Rosa acicularis Lindl.	Rosaceae	Rosa	80.0 ± 8.1	11.3 ± 4.6	69.0 ± 3.9	13	Jl, Au, Ma
Spartina gracilis Trin.	Poaceae	Rosa	73.6 ± 6.0	38.3 ± 5.3	21.0 ± 4.5	18	Ap,Jl,Oc,Ma
Aster laevis L.	Asteraceae	Rosa	60.5 ± 14.3	21.7 ± 10.0	24.8 ± 10.6	7	Ap, Jn, Au
Calamagrostis inexpansa A. Gray	Poaceae	Rosa	44.8 ± 13.0	6.5 ± 3.9	21.0 ± 10.2	5	Ap

[a]Each value is the mean (± SE) percentage of roots (50 observed/root system) with AMF-colonization.

[b]Each value is the mean (± SE) percentage of roots (50 observed/root system) with arbuscules.

[c]Each value is the mean (± SE) percentage of roots (50 observed/root system) with vesicles.

[d]n = the number of root systems examined.

[e]Ap -April, Jn -June, Jl -July, Au -August, Oc -October, 1991, Ma -May, 1992.

low arbuscule frequency in most plants). Arbuscules were rarely seen in roots of *R. acicularis*, and AMF may not form mutualistic associations with this species.

With the exception of *P. nuttalliana*, high PRC was observed in plants of the Poaceae. Colonization in plants of the Asteraceae was either very common (*Grindelia squarrosa* (Pursh) Dunaland *S. arvensis*), or relatively infrequent (members of the *Aster* genus). Plants from the usually non-mycotrophic families Chenopodiaceae and Triglochinaceae had few colonized roots, with the exception of *Atriplex patula.*

3. MPN of AMF-propagules

AMF-colonization was observed in few bioassay plants inoculated with soil from the Salicornia or Puccinellia zones. MPNs were high in the Hordeum, Agropyron, and Rosa zones (Table 4). MPNs were greater in the Agropyron and Hordeum zones than in the Rosa zone, but the significance of this difference is difficult to assess without knowledge of spatial variation in propagule density within vegetation zones.

4. Phenology of AMF-infection

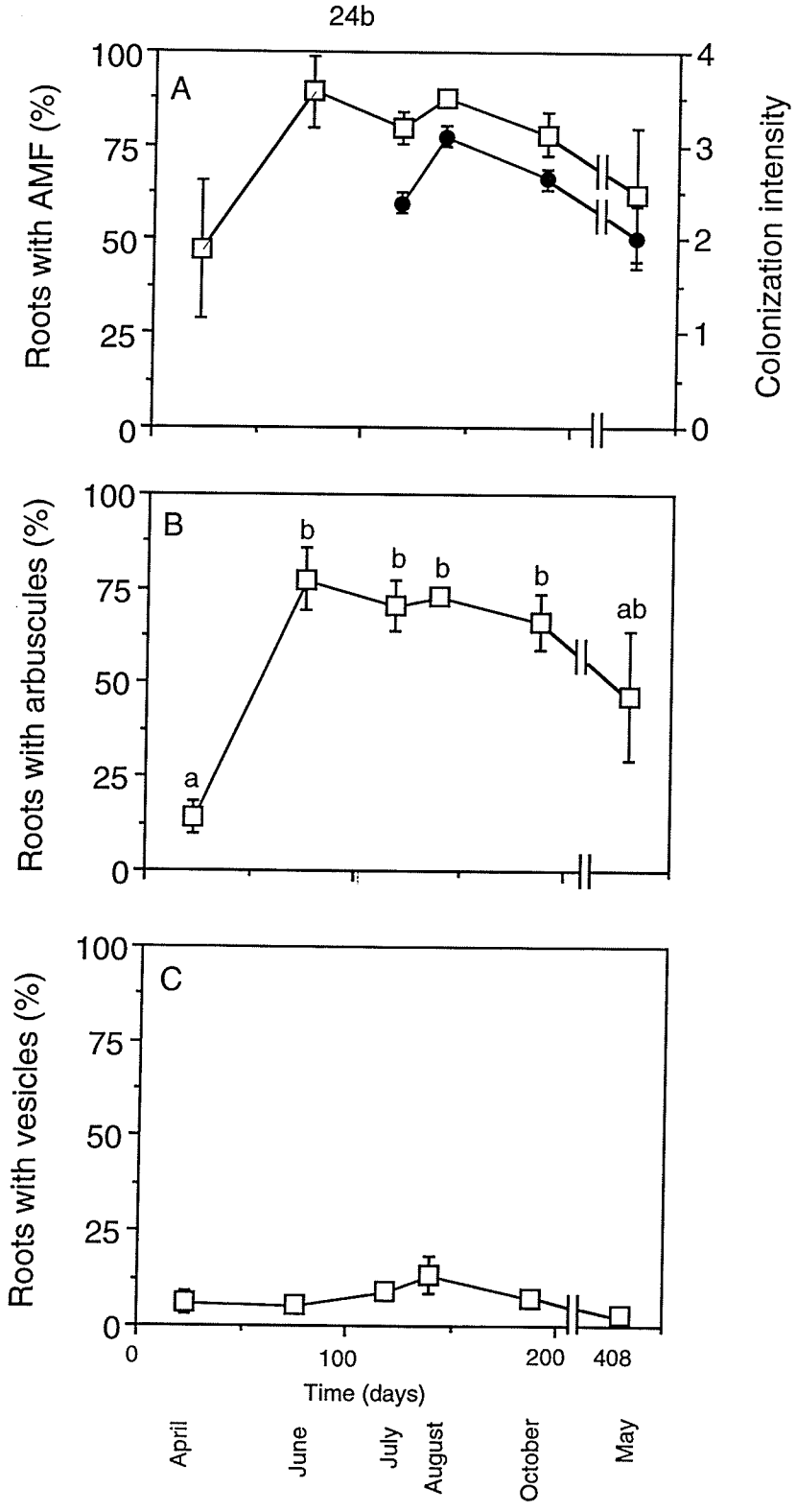
a). Hordeum zone

Young roots of *H. jubatum* were present in April, and throughout the growing season. There were no significant differences among sampling times in the PRC of *H. jubatum* (Figure 3), but variability of PRC was highest in early spring (April, 1991 and May, 1992). CI was largest in August, but not significantly larger than in other months.

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

Vegetation zone	Bait plant	mycorrhizal inoculum (MPN/g of soil)
Rosa	*A. trachycaulum*	4652
Rosa	*S. gracilis*	4467
Agropyron	*A. trachycaulum*	15332
Agropyron	*C. inexpansa*	10380
Hordeum	*A. trachycaulum*	6630
Hordeum	*H. jubatum*	11398
Puccinellia	*A. trachycaulum*	0.73
Puccinellia	*P. nuttalliana*	0
Salicornia	*A. trachycaulum*	0.69
Salicornia	*S. rubra*	0

Figure 3. Mycorrhizal colonization and activity in *H. jubatum*. Each symbol is the mean ± SE of at least four samples. Each sample consisted of 50 root pieces. Day 0 = April 1, 1991. (A) Percentage of root pieces that had AMF-colonization (*open square*), and the mean colonization intensity (*closed circle*) 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.


24b
A
Roots with AMF (%)
Colonization intensity
100
75
50
25
0
4
3
2
1
0
B
Roots with arbuscules (%)
a
b
b
b
b
ab
C
Roots with vesicles (%)
0
100
200
408
Time (days)
April
June
July
August
October
May

PRA was smaller in April than at any other time except May, 1992. PRV remained small throughout the growing season.

D. stricta, also common in the Hordeum zone, had a different seasonal pattern of root growth than *H. jubatum*. Few roots of *D. stricta* were seen in April or May. The PRC was significantly larger in July than in August or October (Figure 4). Residuals were highly skewed, and the Kruskal-Wallis test did not confirm this difference, which suggests that it may not be reliable. However, there was also a significant late-summer decline in the PRA. PRA was larger in June and July than in August and October ($p<0.01$). Significant differences were not observed in CI or PRV of *D. stricta*.

b). Agropyron zone

There were no significant differences in PRC or CI of *A. trachycaulum* throughout the sampling period (Figure 5). In contrast, there was a complex pattern of arbuscule and vesicle production. PRA was small in April, peaked in June, declined gradually in July and August, and was large but variable in October and May. PRV was larger in April and August than in June, October, or May. There was a significant negative correlation between the proportion of colonized roots with arbuscules and the proportion of colonized roots with vesicles ($r=-.75$). This correlation was not seen in other host species.

S. arvensis was also common in the Agropyron zone. It had a similar phenology of root growth as *D. stricta*; there were few roots in April or May, but abundant roots throughout the rest of the growing season. PRC and CI were both large in July, August, and October, and significantly smaller in May (Figure 6). A similar pattern was observed in PRA, although PRA was not significantly smaller in May. PRV was significantly larger in August than in May.

Figure 4. Mycorrhizal colonization and activity in *D. stricta.* Each symbol is the mean ± SE of at least four samples. Each sample consisted of 50 root pieces. Day 0 = April 1, 1991. (A) Percentage of root pieces that had AMF-colonization (*open square*), and the mean colonization intensity (*closed circle*) 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.

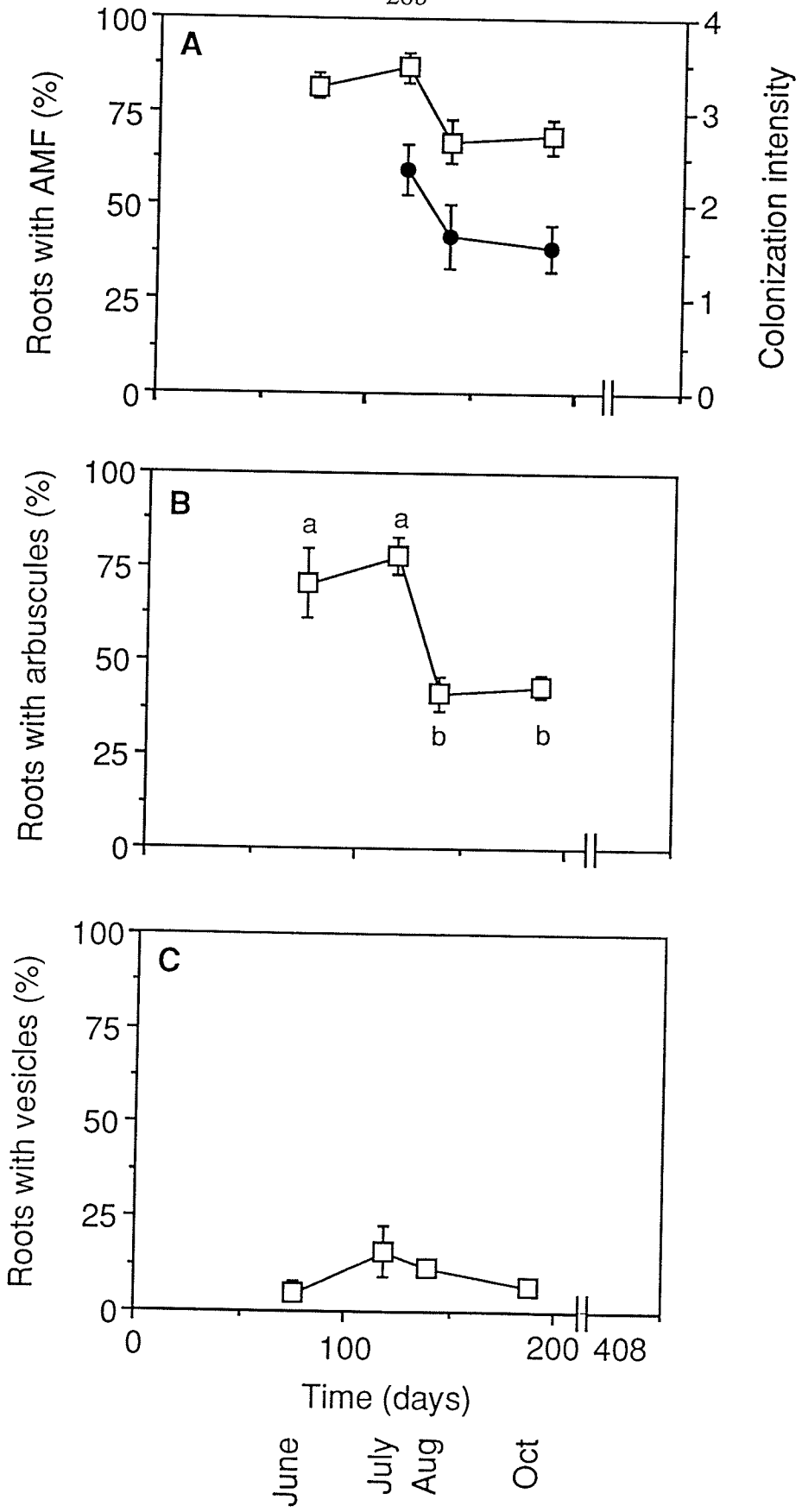

A
Roots with AMF (%)
Colonization intensity
100
75
50
25
0
4
3
2
1
0
B
Roots with arbuscules (%)
a
a
b
b
C
Roots with vesicles (%)
0
100
200
408
Time (days)
June
July
Aug
Oct

Figure 4. Mycorrhizal colonization and activity in *A. trachycaulum.* Each symbol is the mean ± SE of at least four samples. Each sample consisted of 50 root pieces. Day 0 = April 1, 1991. (A) Percentage of root pieces that had AMF-colonization (*open square*), and the mean colonization intensity (*closed circle*) 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.

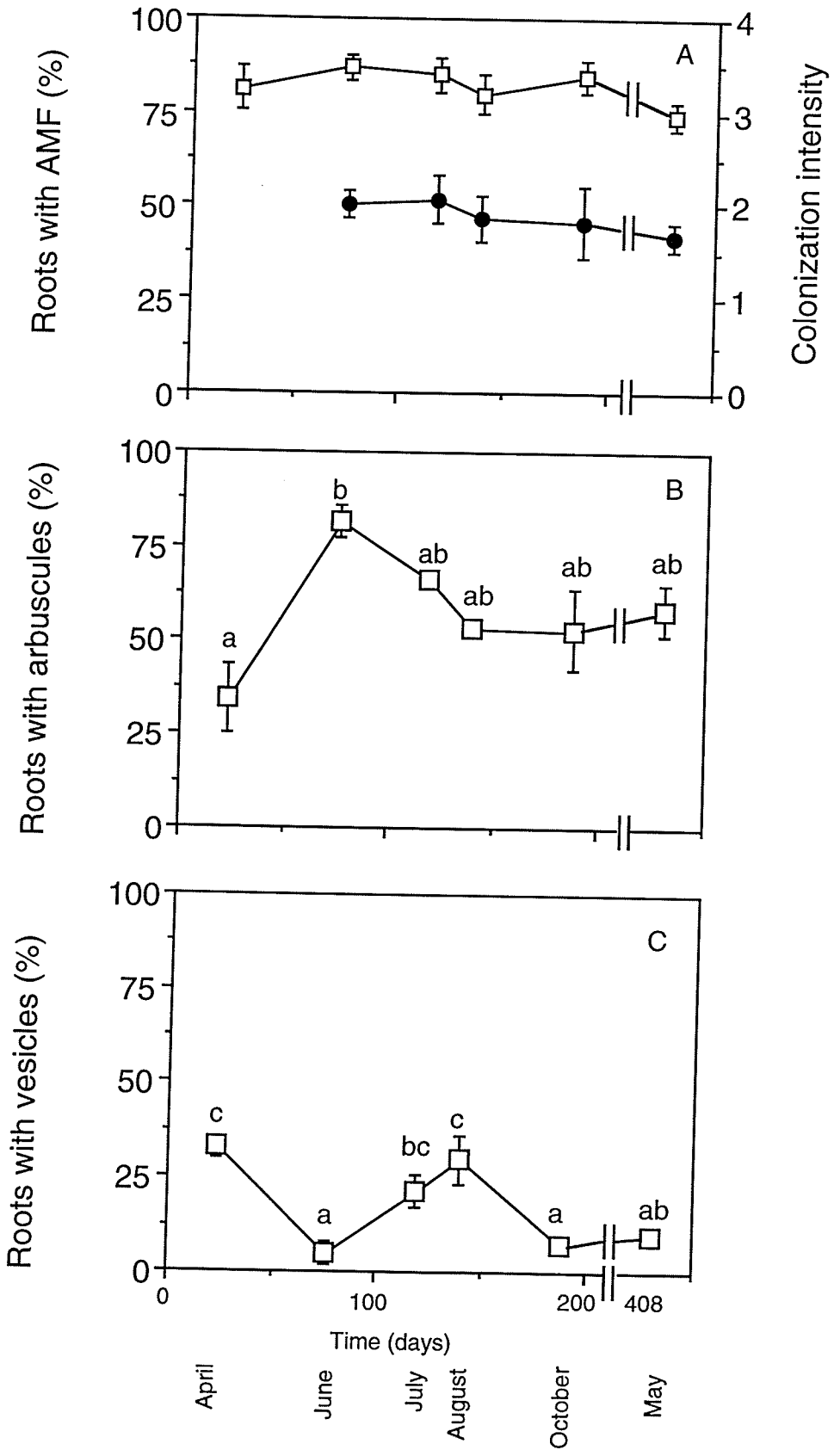
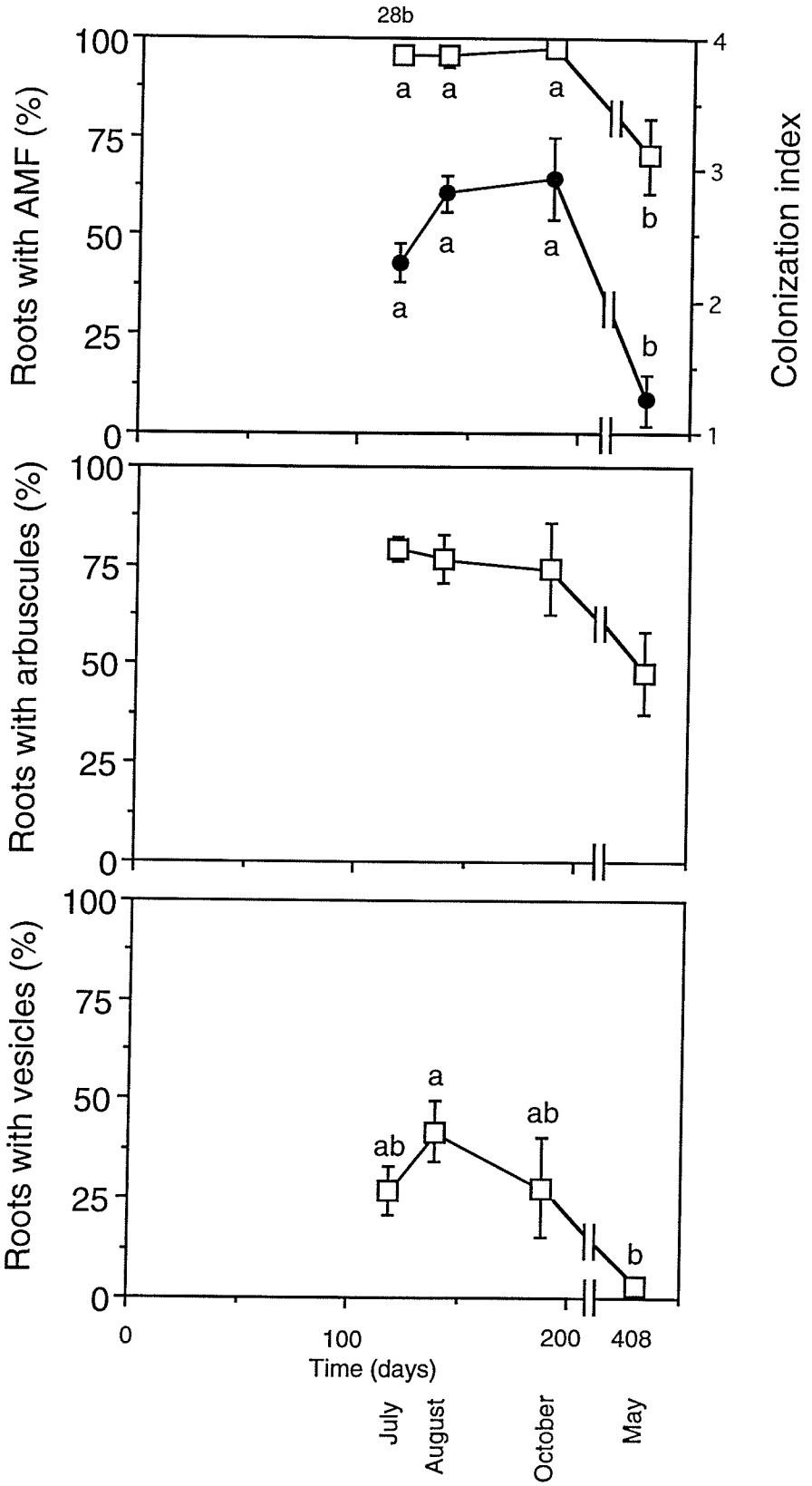

Roots with AMF (%)
Colonization intensity
A
100
75
50
25
0
4
3
2
1
0
Roots with arbuscules (%)
B
a
b
ab
ab
ab
ab
Roots with vesicles (%)
C
c
a
bc
c
a
ab
0
100
200
408
Time (days)
April
June
July
August
October
May

Figure 6. Mycorrhizal colonization and activity in *S. arvensis*. Each symbol is the mean ± SE of at least four samples. Each sample consisted of 50 root pieces. Day 0 = April 1, 1991. (A) Percentage of root pieces that had AMF-colonization (*open square*), and the mean colonization intensity (*closed circle*) 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.


28b
Roots with AMF (%)
Colonization index
Roots with arbuscules (%)
Roots with vesicles (%)
100
75
50
25
0
4
3
2
1
a
a
a
b
a
a
a
b
a
ab
ab
b
0
100
200
408
Time (days)
July
August
October
May

c). Rosa zone

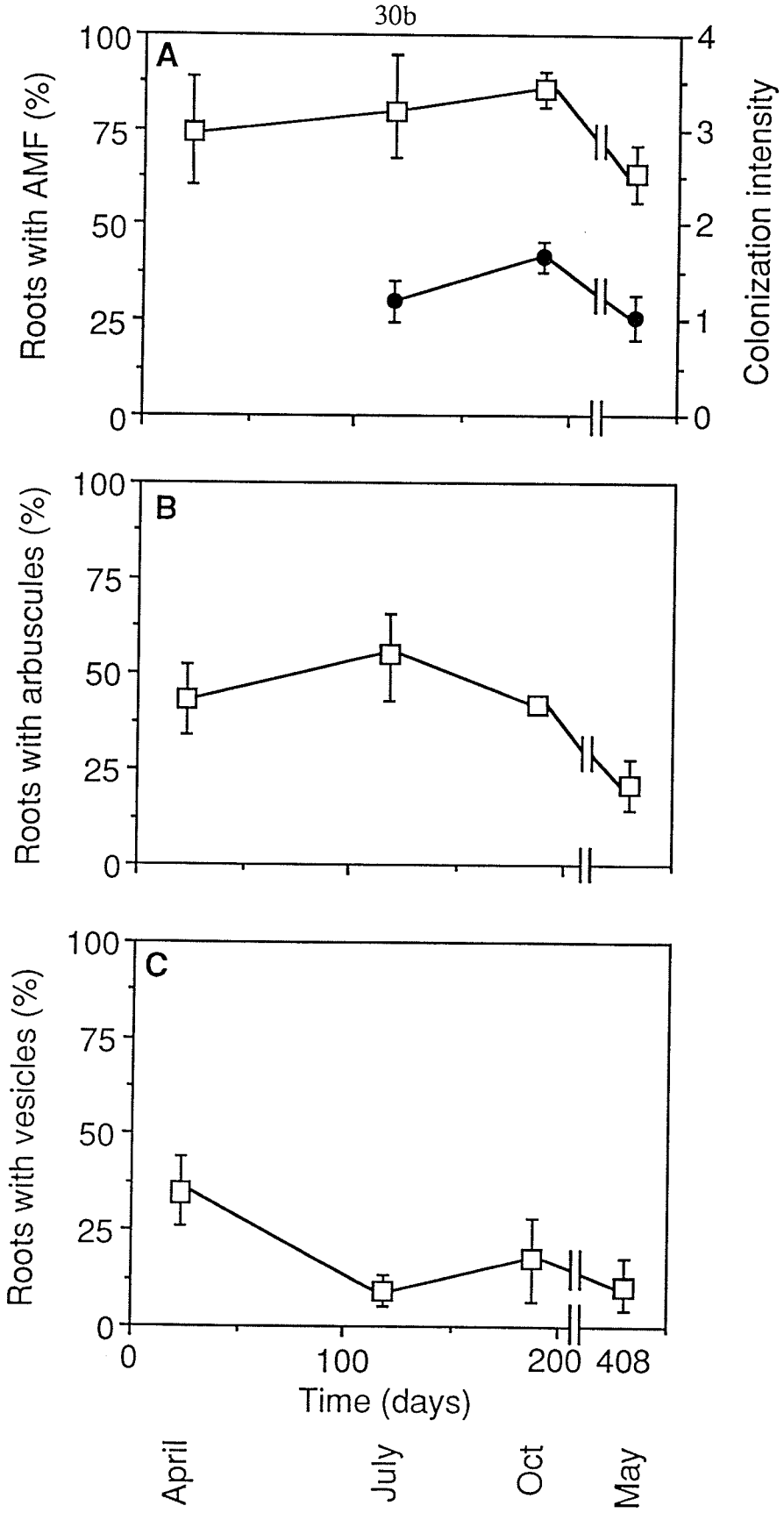
The phenology of colonization is more difficult to interpret in the Rosa zone, which had low levels of soil salinity. It was difficult to acquire adequate numbers of roots of *Rosa acicularis* throughout the growing season, especially in the early spring. I collected roots of *S. gracilis* in April, July, October, and May, and roots of *Aster laevis* L. in April, June, and August. From these collections it is evident that PRC was small in *A. laevis*, and PRA and PRV were similar (Table 3). In contrast, AMF-colonization in *S. gracilis* was frequent from April to October, and in the following May (Figure 7). CI remained low throughout the growing season. PRA and PRV of *S. gracilis* were variable throughout, and no significant differences were observed. However, the proportion of colonized roots that had vesicles was higher in April than in May or July.

F. Discussion

1. Exclusion of AMF from extremely saline soil

I observed a zonation of AMF. AMF were extremely infrequent in roots from the highly saline Puccinellia and Salicornia zones (the "Pucc-Sal" non-mycorrhizal zone), despite their prevalence in the Hordeum, Agropyron and Rosa zones (the "Ho-Ag-Ro" mycorrhizal zone). The exclusion of AMF from the Pucc-Sal zone was also apparent in the soil bioassay; AMF-propagules were absent in soil from the Pucc-Sal zone, but were present in large numbers in the Ho-Ag-Ro zone. The exclusion of AMF from the Pucc-Sal zone appears to be due to the protracted period of high soil salinity. The mean soil water salinity in the Ho-Ag-Ro zone approached seawater (3.4 mg salt/mL water) only in August, whereas it rose above this level in the Pucc-Sal zones in June, July, and August.

Figure 7. Mycorrhizal colonization and activity in *S. gracilis*. Each symbol is the mean ± SE of at least four samples. Each sample consisted of 50 root pieces. Day 0 = April 1, 1991. (A) Percentage of root pieces that had AMF-colonization (*open square*), and the mean colonization intensity (*closed circle*) 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.


A
B
C
Roots with AMF (%)
Colonization intensity
Roots with arbuscules (%)
Roots with vesicles (%)
100
75
50
25
0
4
3
2
1
0
0
100
200
408
Time (days)
April
July
Oct
May

The low levels of available phosphorus in the Pucc-Sal zone would be expected to lead to frequent AMF-colonization; in contrast, the relatively high levels of phosphorus in the Ho-Ag-Ro zone would be expected to result in lower levels of AMF-colonization. Since the opposite pattern was observed, it appears that soil salinity is more important than soil phosphorus in the regulation of AMF-colonization and activity in this community. Differences in the duration of soil saturation may also have affected AMF-colonization. In the spring, soil in the Pucc-Sal zone may remain saturated longer than in the Ho-Ag-Ro zone. This could inhibit AMF-colonization. The importance of soil moisture may be minimal however, because soil of the Hordeum zone appeared to remain saturated for a similar length of time in the spring as did the Puccinellia 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 10x more concentrated than calcium in saline springwater in the Overflowing River Area (van Everingden 1971; McKillop et al. 1992). The high concentration of Ca I observed may have been caused by dissolution of Ca-containing minerals by Na-rich groundwater (Javor 1989) in the spring. Subsequent leaching of Na may then have led to predominance of Ca over Na in the soil exchange-complex.

It is possible that the lack of AMF in the Pucc-Sal zone was caused by the absence of suitable hosts, since most plants in this zone are from non-mycotrophic families. However, greenhouse-grown *P. nuttalliana* seedlings were moderately well-colonized by AMF after exposure to non-sterile soil from the Agropyron zone (Chapter V). This question is further addressed in Chapter III.

Another explanation for the lack of AMF in extremely saline soil is that highly salt-tolerant species of AMF were not present at the site. AMF-diversity has been found to decrease with increasing latitude (Koske 1987), and the Overflowing River area may be near the northern limit of the distribution of AMF. Indeed, I found very low AMF

diversity. In numerous spore-extractions from soil from the salt pan described here, virtually all of the spores were identified as *Glomus etunicatum* Beck.&Gerd. Pond *et al.* (1984) identified 6 species from 3 genera of AMF in soil samples from salt pans in southwestern USA. Increased diversity of AMF may explain their observation of AMF colonization in extremely saline soil (>60,000 ppm Na). Since Pond *et al.* (1984) did not quantify AMF-activity, it is also possible that the colonization observed was dormant and had been active during a time when the soil was less severely saline.

In a study of AMF-colonization in salt playas in Utah, Kim and Weber (1985) found a similar pattern as in our study. AMF were common in roots of *D. stricta* (< 5000 ppm Na in soil) but rare in *Salicornia pacifica* (>10,000 ppm Na in soil). The specific ion composition in salt pan soil, rather than the concentration of Na, may be important in the exclusion of AMF from certain soils. This could explain the discrepancies in the levels of Na that appear to inhibit AMF in our study and the studies of Kim and Weber (1985) and Pond *et al.* (1984).

The distribution of AMF in salt-marshes appears to be more complex than the distribution in inland salt pans. In a study of AMF-distribution in a salt marsh in the Netherlands, AMF-colonization was not restricted to particular vegetation zones (Rozema *et al* 1986). In their study, tidal inundation may have prevented the rise in soil salinity to extreme levels. A subsequent study showed that AMF may be inhibited in lower areas of salt marshes, where tidal inundation is prolonged (van Duin *et al* 1989).

2. Inhibition of AMF in spring

The most prominent seasonal pattern in AMF-activity was a low level (small PRA, large PRV, or both) in early spring (April, 1992 or May, 1993). This was observed in *H. jubatum* (small PRA in April) and *A. trachycaulum* (small PRA and large PRV in April). Decreased activity in the spring was less evident in *S. arvensis* and *S. gracilis*, although

PRC in *S. arvensis* decreased in May, and PRV in *S. gracilis* was large in April. Few roots of *D. stricta* were found in April or May, and these roots were almost always non-mycorrhizal.

Low levels of AMF-activity or colonization in early spring have often been observed (e.g. Sutton, 1973, Jakobsen and Nielsen, 1983, Cade-Menun *et al.*, 1991). There is strong experimental evidence that AMF-colonization and host benefit from AMF are absent at low soil temperatures (Furlan and Fortin 1973; Daniels *et al.* 1984; Smith and Roncadori 1986). However, AMF have also been shown to stimulate plant growth at cool temperatures (Volkmar and Woodbury, 1989, Bentivenga and Hetrick, 1992). In addition, several studies of natural systems 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). This inconsistency may be related to differences in cold tolerance among species of AMF.

Cold intolerance of AMF, however, may not be the prime reason for spring-inhibition of AMF at our site, since AMF-colonization and AMF-activity in October did not decline from summer-levels. Air temperature is relatively similar in the September-October and April-May periods (Figure 1), but AMF-activity was in general much greater in the fall than in the spring. Another possible explanation for the decline in AMF-activity in the spring is that the long winter led to low levels of AMF-propagules (colonized roots, spores, and hyphae). This, combined with rapid root growth in the spring may have led to low AMF-activity. Saturated soil in May probably also contributed to low levels of AMF-activity.

3. AMF-phenology and host-phenology

One enduring problem in AMF-research has been the difficulty in demonstrating mycorrhizal benefit in the field (Fitter, 1991). One strategy that has recently been tested

(Sanders and Fitter, 1992a and b; Mullen and Schmidt, 1993) has been to determine if the time of peak arbuscule formation coincides with the time of peak P uptake. If AMF are active during times of little P-uptake in the host, then the potential for host benefit is decreased, because of the lack of "return" on the host's "investment" in the association.

Studies by Schwarz and Redmann (1990) and Jones (1991) indicate that the peak period of growth and flower initiation in halophytic grasses in the northern prairies is spring (late May to early July). This may also be the peak period of P-uptake for these grasses, although this remains to be confirmed. At our site PRA was largest in June in both *H. jubatum* and *A. trachycaulum*, thus indicating the potential for mycorrhizal benefit to these hosts. Ballard (1988, cited by Ungar, 1991, p. 65) also observed rapid AMF-colonization of *H. jubatum* during the peak period of growth in an inland salt pan.

The potential benefit at other times is difficult to assess. In inland salt pans, plants would be expected to be 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 plants to retain the capability for root growth and nutrient uptake (via roots or mycorrhizae) throughout the growing season. Studies of the phenology of root growth in saline soil would be valuable, since there is apparent variation among halophytes in the phenology of shoot growth (Schwarz and Redmann 1990; Ungar 1991).

4. AMF-phenology in different vegetation zones

AMF phenology was not identical in the Hordeum and Agropyron zones. Both *A. trachycaulum* and *S. arvensis* (Agropyron zone) had increased PRV in August, whereas PRV of *H. jubatum* or *D. stricta* (Hordeum zone) was not significantly different at any point in the growing season. Since the same species of AMF appeared to dominate both zones, this difference was unlikely to have been caused by differences in the species of

AMF, but was probably caused by differences in host salt-tolerance. Increased root death in August, the time of peak soil-salinity, may have led to increased vesicle production in *A. trachycaulum* and *S. arvensis*, whose moderate level of salt-tolerance excludes them from the Hordeum zone. The AMF-species present at Overflowing River therefore appear to be unaffected by seasonal peaks of soil salinity, unless the host plant is affected.

In conclusion, it is clear that *G. etunicatum* has considerable salt tolerance. This fungus may have a potential use in the reclamation of saline land, since it is able to survive long-term exposure to saline soil. However, there may be limits to its salt-tolerance; it is not found in highly saline soil colonized by plants such as *P. nuttalliana.*

Chapter III

Relationship between vegetation zonation and mycorrhizal zonation at an inland salt pan

A. Abstract

Chapter 2 illustrated that AMF are common in moderately saline soil (the Hordeum zone) in an inland boreal salt pan in north-central Manitoba, but are absent from extremely saline soil (the Puccinellia zone). It was unclear, however, if this absence was due to the lack of suitable plant hosts, or to increased soil salinity. To clarify this issue, AMF-colonization and vegetation composition were examined along a transect between the Puccinellia and Hordeum zones.

As the transect approached the Hordeum zone, the frequency of AMF-colonization of *Puccinellia nuttalliana* increased. This coincided with the appearance of *Distichlis stricta* (a common plant in the Hordeum zone). Arbuscular mycorrhizal fungi did not appear to be dependent on hosts such as *D. stricta*, because few colonized roots of *D. stricta* were observed.

AMF-colonized seedlings of *P. nuttalliana* were transplanted into both vegetation zones to test the ability of AMF to maintain colonization over a growing season in the field. After one growing season, little AMF-colonization was observed in transplants in the Puccinellia zone, but transplants in the Hordeum zone had moderate levels of AMF-colonization.

B. Introduction

Vegetation zonation is often conspicuous in the presence of gradients of soil salinity; for example, coastal salt marshes (Odum, 1988) and inland salt pans (Ungar,

1974) typically have pronounced zonation. In Chapter II it was suggested that there are distinct mycorrhizal zones in inland salt pans. Arbuscular mycorrhizal fungi are common in non-saline or moderately saline soil (dominated by *Hordeum jubatum*), but are absent in an adjacent vegetation zone dominated by *Puccinellia nuttalliana.*

The previous study (Chapter II) did not determine if boundaries between vegetation zones coincided with boundaries between mycorrhizal zones. If they coincide, then mycorrhizal zonation is probably caused by changes in the suitability of plant species to support mycorrhizal colonization. Most of the plant species in extremely saline soil belong to the non-mycotrophic (Newmann and Reddell, 1987) families Chenopodiaceae and Triglochinaceae, and *P. nuttalliana*, a member of the usually-mycorrhizal Poaceae family, tends to be less-well colonized than other halophytic grasses (Chapter V, Table 10).

If mycorrhizal and vegetation boundaries do not coincide, then mycorrhizal zonation is probably caused by inability of AMF to tolerate the extreme levels of soil salinity in the Puccinellia zone. There were 2 objectives of this study: (*i*) to determine if the boundary between the Hordeum and Puccinellia zones coincides with the boundary between the mycorrhizal and non-mycorrhizal zones; (*ii*) to test the ability of AMF to maintain colonization in the Puccinellia and Hordeum zones. This was accomplished by the transplantation of mycorrhizal seedlings of *P. nuttalliana* into each vegetation zone.

C. Methods

1. AMF-colonization in the Puccinellia zone

In 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 Hordeum zone (see Table 1 for a description of these zones). This study took place in

the same salt pan as was described in Chapter II.

Beginning at the point of the transect closest to the Salicornia zone, soil and root samples were taken at 2m intervals. Percent cover of the vegetation in a 0.5 x 0.5m quadrat was also recorded at each sampling point. At points of the transect less than 4m from the beginning of the Hordeum zone (marked by the presence of *H. jubatum* or *Distichlis stricta*), the sampling interval was 1m. Soil samples were taken to measure soil salinity. Soil was dried, extracted with 5 volumes of water, conductivity of the extract was measured, and the salinity of soil water was calculated (Chapter II). Root samples of *P. nuttalliana* were collected at each point of the transect, and at the fringe of the Hordeum zone, roots of *D. stricta* were also collected. *H. jubatum* was not sampled because it was uncommon near the boundary of the Hordeum and Puccinellia zones. *D. stricta* is very common in the Hordeum zone, and the boundary area between the Hordeum and Puccinellia zones is typically a mixture of *P. nuttalliana* and *D. stricta.*

Methods of staining of roots, and of quantification of AMF-activity are described in Chapter II. The percentage of root pieces with AMF-colonization (PRC) was determined for each root sample.

2. Transplant experiment

In June, 1992, an additional transect was established in the Puccinellia zone, perpendicular to the 5 other transects. This transect was 2m from the Salicornia zone and followed the contours of the boundary between the Salicornia and the Puccinellia zones. At each of 10 points (2m apart) along this transect two groups of 5 seedlings of *P. nuttalliana* were transplanted. These seedlings (27 days old) had been germinated in 10 cm pots containing autoclaved potting soil (see Chapter VI, Section C-2 for

composition) and 10 g of AMF-inoculum in the form of soil collected from the Hordeum zone in August, 1991. Seedlings from 2 pots were transplanted at each transect point; one contained untreated inoculum, and the other contained autoclaved (1 hr at 121°C) inoculum (*i.e.* with AMF killed). A similar transect, with transplanted *P. nuttalliana* was established in a nearby salt pan in a zone dominated by *H. jubatum* and *D. stricta.*

Plants were harvested 75 days later, in late August. A subsample of the roots was preserved in 50% ethanol, stained with trypan blue (Chapter II), 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°C and weighed.

D. Results and Discussion

1. Plant and AMF distribution along the transects

To simplify interpretation, percent cover results are only presented for *P. nuttalliana* and *D. stricta* (Figures 8 and 9). *Suaeda maritima* and *Triglochin maritima* were also common throughout the Puccinellia zone, although often infrequent (5-20% cover). In all of the transects except transect 5, the percent cover of *P. nuttalliana* decreased towards the end of the transect, as *D. stricta* began to appear. In transects 1,2, and 4 the rise in percent cover of *D. stricta* coincided with the rise in AMF colonization (Figures 8 and 9). It should be emphasized, though, that figures 1 and 2 display PRC of *P. nuttalliana*, not *D. stricta.* Few colonized roots of *D. stricta* were observed in this

Figure 8. Percent plant cover and percent of root pieces colonized by AMF (PRC) at points along transects. The x-axis indicates distance from the starting point of the transect (near the boundary of the Salicornia and Puccinellia zones). (A), transect 1; (B), transect 2; (C), transect 3. *Closed triangles* , percent cover of *P. nuttalliana*; *closed circles*, percent cover of *D. stricta*; *open squares*, PRC.

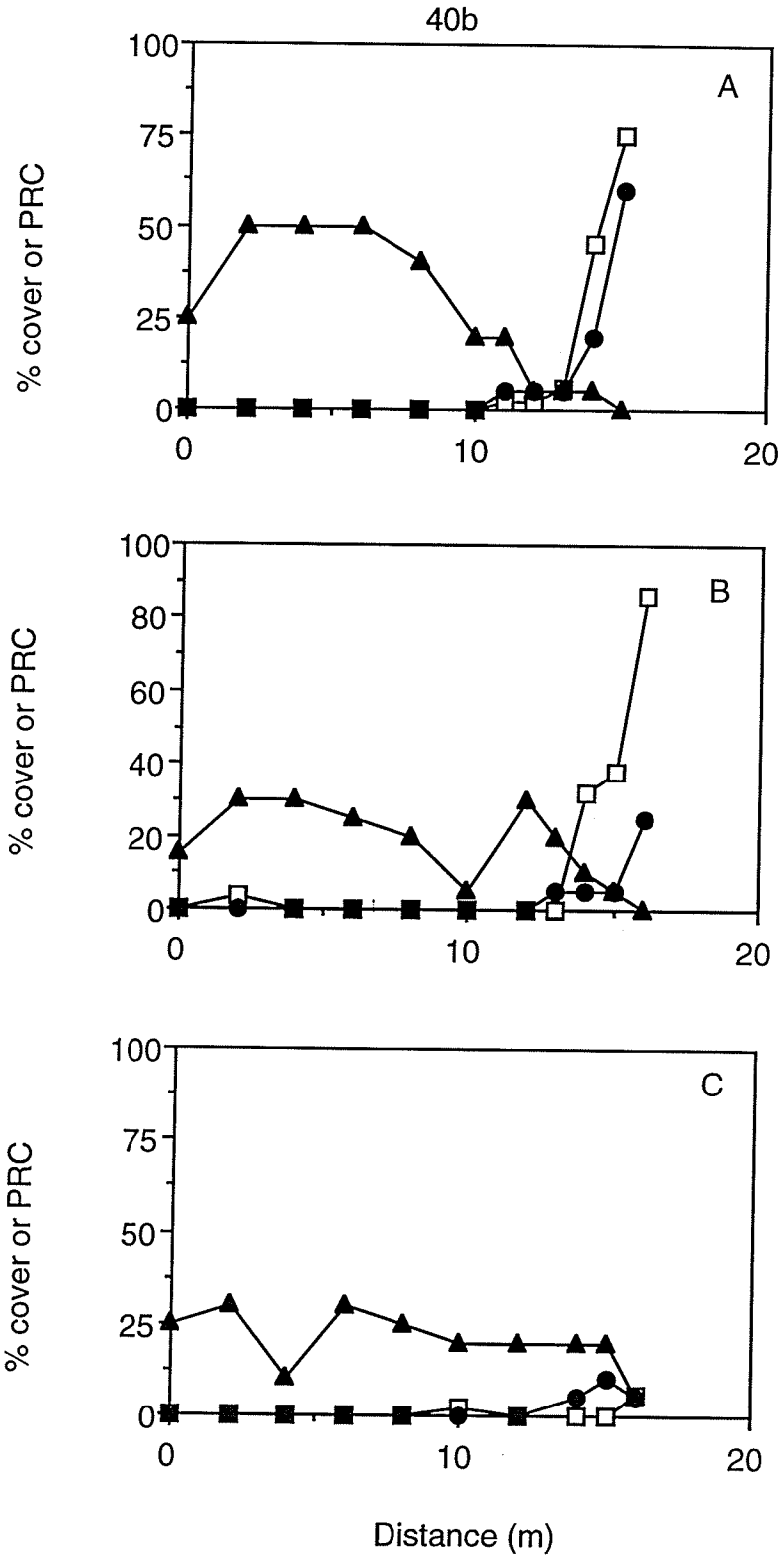
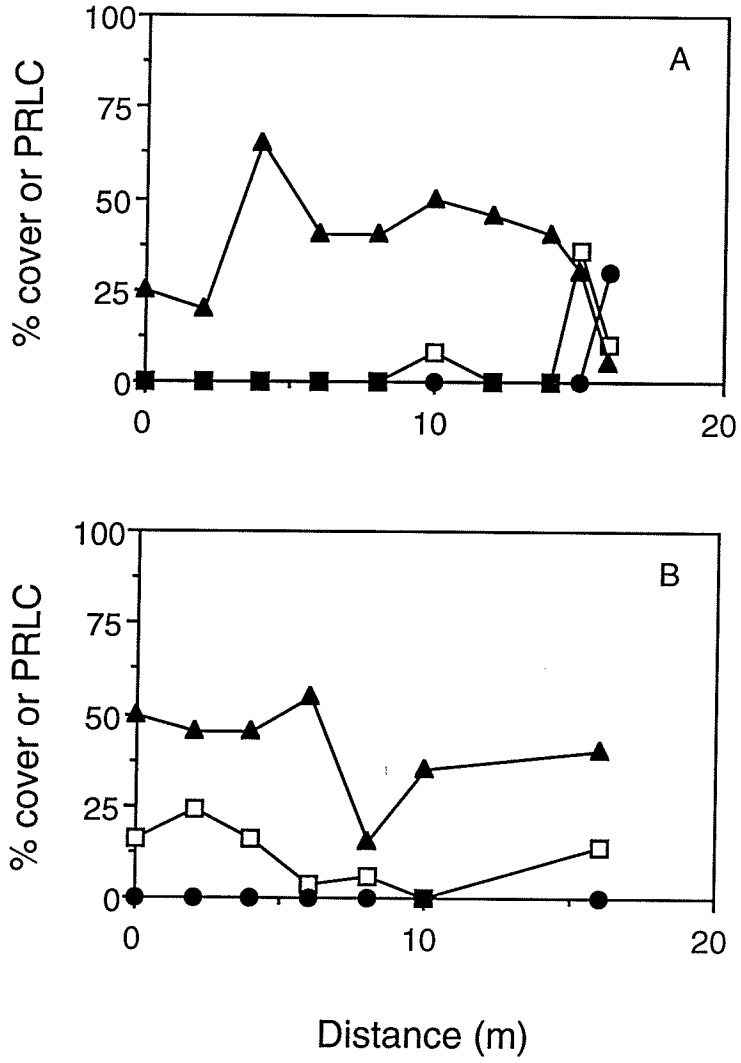

40b
A
B
C
% cover or PRC
Distance (m)
100
75
50
25
0
80
60
40
20
0
10
20

Figure 9. Percent plant cover and percent of root pieces colonized by AMF (PRC) at points along transects. The x-axis indicates distance from the starting point of the transect (near the boundary of the Salicornia and Puccinellia zones). (A), transect 4; (B), transect 5. *Closed triangles* , percent cover of *P. nuttalliana*; *closed circles*, percent cover of *D. stricta*; *open squares*, PRC.


100
75
50
25
0
% cover or PRLC
A
0
10
20
100
75
50
25
0
% cover or PRLC
B
0
10
20
Distance (m)

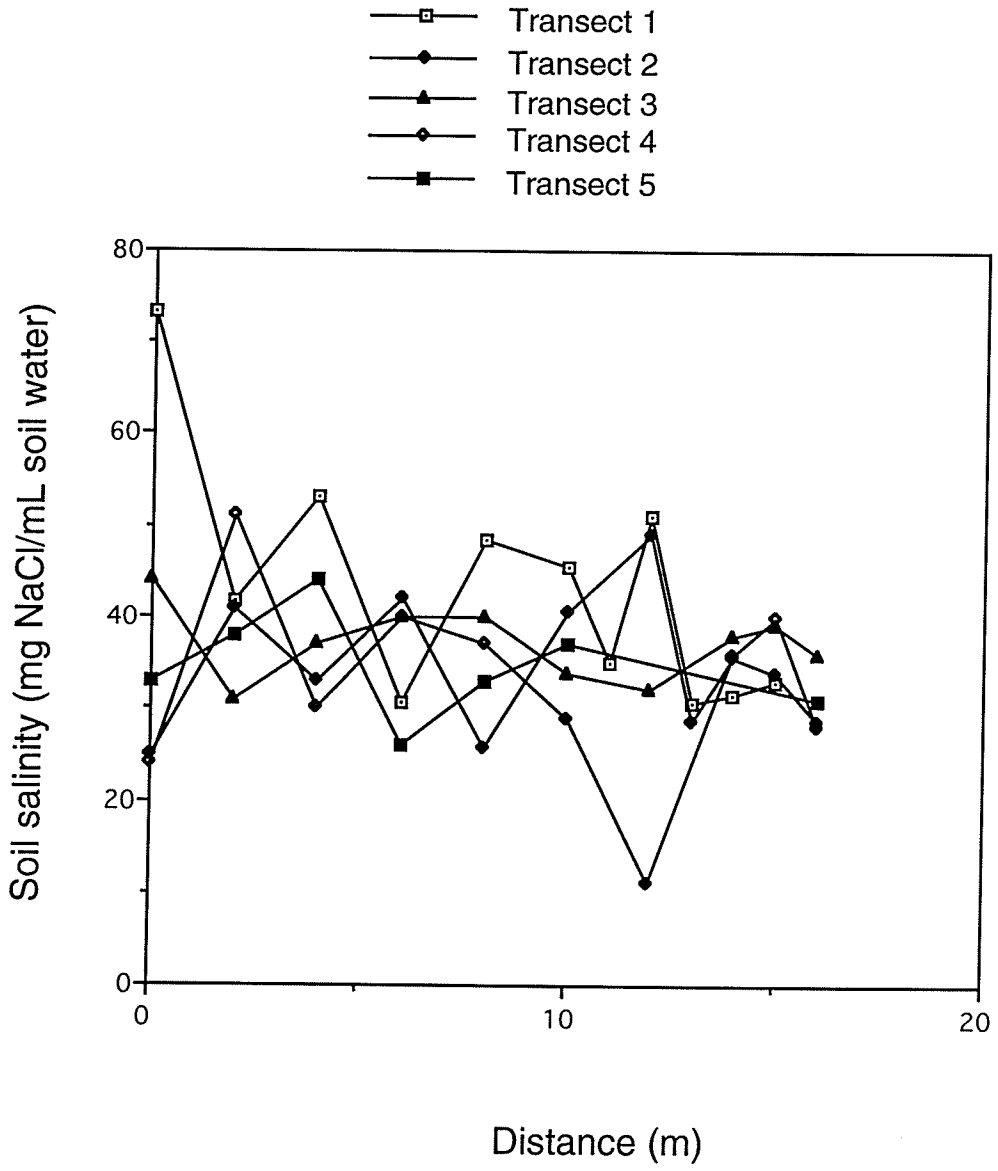
study, perhaps because of the small size of the root system of *D. stricta* at this time of year. There is often a "lag-phase" in AMF-colonization in early stages of growth of the root system (Gazey *et al.* 1992).

The presence of AMF as the Hordeum zone is approached, then, is not due to the presence of 'suitable hosts' (*i.e. D. stricta*). Soil salinity is the most likely agent, then, for the exclusion of AMF from the Puccinellia zone. Soil salinity did not dramatically decrease as the Hordeum zone was approached, and *D. stricta* began to be represented (Figure 10), but the extreme variation evident throughout the Puccinellia zone decreased substantially in the last 3 sampling points of each transect. This coincided with the appearance of *D. stricta* and AMF, indicating that the absence of these organisms throughout the bulk of the Puccinellia zone is caused by their inability to survive extreme spatial variation in soil salinity. It is also possible that physiological responses of halophytes to this extreme variation may lead to inhibition of mycorrhizal colonization; further research on the relationship between salinity and AMF-colonization of halophytes such as *P. nuttalliana* would be valuable.

2. Transplant experiment

P. nuttalliana seedlings that received autoclaved inoculum were colonized by indigenous AMF when transplanted to the Hordeum zone. PRLC was not significantly different in plants in the Hordeum zone that were previously exposed to untreated or autoclaved inoculum. Moderate levels of colonization (10-20% PRLC) were observed in plants transplanted into the Hordeum zone. PRLC was very low (<5%) in roots of plants with autoclaved or untreated inoculum that were transplanted into the Puccinellia

Figure 10. Soil salinity at points along transects 1-5. The x-axis indicates distance from the starting point fo the transect (near the boundary of the Salicornia and Puccinellia zones).


Transect 1
Transect 2
Transect 3
Transect 4
Transect 5
Soil salinity (mg NaCl/mL soil water)
80
60
40
20
0
0
10
20
Distance (m)

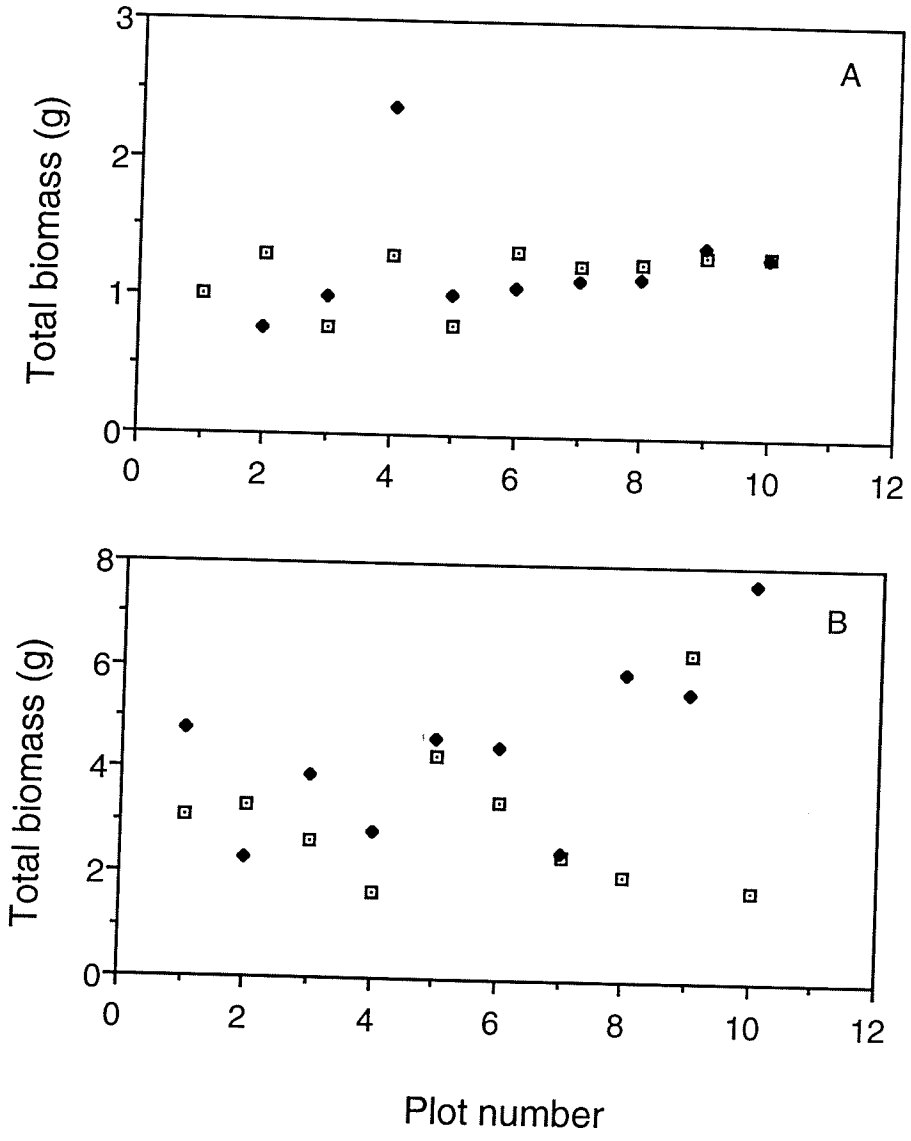
zone. AMF, therefore, appear to be unable to sustain colonization in the Puccinellia zone.

In the Hordeum-Distichlis zone total biomass (Figure 11) was higher (paired t-test) in plants with untreated inoculum than in plants with autoclaved inoculum, although the significance of this difference was marginal ($p<0.08$). Because of the similarity in PRLC in these 2 groups, it is unclear if the difference was caused by AMF, or other microorganisms in the soil inoculum. In the Puccinellia zone, biomass was similar in plants that received autoclaved or untreated inocula (Figure 11).

In conclusion, the absence of AMF colonization in the bulk of the Puccinellia zone arises because of inability of AMF to maintain colonization in the Puccinellia zone, perhaps because of extreme heterogeneity in salinity. This agrees with a previous study of plant growth in these sites, which concluded that plants that are not highly salt-tolerant are excluded from the Puccinellia and Salicornia zones because of extreme variability in soil salinity (Jones, 1991).

The coincident rise in AMF-colonization (of *P. nuttalliana*) and % cover of *D. stricta* illustrate that the boundaries between vegetation zones and mycorrhizal zones can be very close. This correspondence does not appear to originate from AMF host-preference, but is caused by a similar inability of AMF and plants such as *H. jubatum* and *D. stricta* to survive extreme variation in soil salinity. It is also possible that AMF are partially responsible for the decline of *P. nuttalliana* at the boundary between the Hordeum and Puccinellia zones. Arbuscular mycorrhizal fungi suppress the growth of *P. nuttalliana* in greenhouse experiments (Chapter IV).

Figure 11. Total biomass of transplants. (A) Puccinellia zone; (B) Hordeum zone. (*open squares*), autoclaved inoculum; (*closed diamonds*), untreated inoculum.


A
B
Total biomass (g)
Total biomass (g)
Plot number
0
1
2
3
4
6
8
10
12

Chapter IV

Effects of soil salinity and arbuscular mycorrhizae on the growth and nutrient uptake of *Agropyron trachycaulum*, *Hordeum jubatum*, and *Puccinellia nuttalliana*

A. Abstract

The aim of this chapter is to determine the effects of AMF on the growth and nutrient uptake of halophytic grasses under varying levels of soil salinity. Mycorrhizal and non-mycorrhizal *Puccinellia nuttalliana*, *Hordeum jubatum*, and *Agropyron trachycaulum* were grown in a growth chamber, and exposed to 4 salinity treatments (0,6,12, and 18 g/L NaCl). Mycorrhizal fungi were introduced via soil inoculum collected from salt pans in the Overflowing River region of north-central Manitoba.

Mycorrhizae depressed shoot and root biomass of all three species at all salinity levels. In a second experiment using soil with greater available phosphorus (P), biomass of mycorrhizal *A. trachycaulum* and *H. jubatum* plants was lower than non-mycorrhizal plants, but the biomass of *P. nuttalliana* was unaffected by mycorrhizae. In the first experiment, mycorrhizal plants of *H. jubatum* had similar levels of total shoot P, and higher P concentration in shoot tissue, as compared with non-mycorrhizal plants. However, the increased P concentration may not have been due to AMF, because increased soil salinity (resulting in decreased shoot biomass) led to a similar increase in P concentration.

P. nuttalliana had less mycorrhizal colonization than *H. jubatum* or *A. trachycaulum*. The percentage of root length colonized by AMF declined as salinity increased in *A. trachycaulum*, but not the other 2 species. Total root length of *P. nuttalliana* was not affected by low levels of salinity, suggesting that in halophytes changes in root architecture may partially compensate for decreased root biomass in

saline soil.

B. Introduction

As shown in Chapter II, AMF are very common in inland boreal salt pans in north-central Manitoba, although they are excluded from extremely saline soils. Other studies (*e.g.* Boullard, 1958, Rozema *et al.*, 1986, Sengupta and Chaudhuri 1990) have also found abundant AMF-colonization in saline soil. Despite these findings, there has been little research on the effects of AMF on the growth and nutrient uptake of halophytes in saline soil.

AMF often improve the growth of glycophytes, usually through improved P-nutrition (Fitter, 1991). Since P deficiency exacerbates salinity stress (Grattan and Grieve, 1992), AMF-driven increases in plant P content would be expected to increase salinity tolerance. Several workers have shown that increased growth in mycorrhizal plants in saline soil is due to improved P nutrition of the host, (Ojala *et al.*, 1983, Poss *et al.*, 1985, Pfeiffer and Bloss, 1988). Duke *et al* (1986), however, found that AMF-colonization improved the salt tolerance of citrus seedlings in the apparent absence of an increase in P nutrition.

Not all research has demonstrated host benefit from mycorrhizae in saline soil. Hartmond *et al.* (1987) observed that AMF either had no effect, or decreased the growth of citrus seedlings in saline soil. A later study also found that chloride levels in citrus leaves were higher in mycorrhizal plants (Graham and Syvertsen, 1989). These contradictory findings may have been caused by variation in the physiology of AMF-species and isolates. A screening study of 38 soil samples from a variety of saline sites yielded positive, negative and neutral effects on host salt tolerance (Pond *et al.*, 1984).

The above studies were concerned with assessing the potential of AMF to improve the salt tolerance of glycophytes. The potential of AMF to affect halophytes has also received attention. Allen and Cunningham (1983) did not observe improved water-relations in mycorrhizal individuals of *Distichlis spicata* (L.) Greene, a halophytic grass. In contrast, Rozema *et al.* (1986) observed increased dry weight and improved water-relations in AMF-colonized *Aster limonium.*

Little is known of the effects of AMF on halophytic grasses other than *D. spicata*, despite the importance of halophytic grasses in inland and coastal saline communities (Chapman, 1960, Ungar, 1974).

The objectives of this chapter are (*i*) to compare the effects of AMF on the growth and nutrient uptake of three halophytic grasses exposed to varying levels of soil salinity, and (*ii*) to examine the effects of increased soil salinity on AMF-colonization. *Puccinellia nuttalliana*, *H. jubatum* and *A. trachycaulum* were chosen because of their importance in Manitoba salt pans (Burchill and Kenkel, 1991), and because they differ widely in their level of salt tolerance. The *Puccinellia* genus is highly salt tolerant (*e.g.* Ewing *et al.*, 1989), *H. jubatum* is moderately salt tolerant (*e.g.* Wang *et al.*, 1992), and *A. trachycaulum*, based on its distribution in the field (Burchill and Kenkel, 1991) has a low level of salt tolerance.

C. Materials and Methods

1. Experimental design

Two greenhouse experiments, with an identical design, are described in this chapter. In each experiment, each plant species was seeded into 32 pots; 16 pots were "mycorrhizal", and 16 were "non-mycorrhizal". Within each of these groups of 16

plants, 4 pots were assigned to each of 4 levels of soil salinity. Since three plant species were studied, there was a total of 96 pots in each experiment.

2. Soil preparation and treatment

a). Experiment 1

In order to simulate conditions in the field, plants were grown in soil collected from a salt pan near Overflowing River, Manitoba. To minimize disturbance of this Ecologically Protected Area, soil was collected from an unvegetated, highly saline area of the salt pan. The soil was autoclaved at 121°C for 90 minutes, and then was extensively washed with water to remove soluble salts. When the conductivity of water overlying the soil had stabilized at appproximately 0.8 dS/m, the soil was mixed with coarse sand (3 sand:1 soil). Tri-calcium phosphate (a relatively insoluble source of phosphorus) was added to the soil to give a final concentration of 10 g/kg. This was added to increase the availability of phosphorus (P) to a level that would support plant growth without suppressing mycorrhizal colonization. Hoagland's solution (1/5 strength -see Appendix B for composition) without P was added to the pots on days 30 and 69 after seeding.

To alleviate P-deficiency symptoms in both mycorrhizal and non-mycorrhizal plants, I supplemented each pot with 2 mg of $NaH_2PO_4.2H_2O$ on day 69. Dilute H_2SO_4 was also added at this time, because soil pH had risen to levels >8.5 during the course of the experiment. After four days of daily acidification, the pH had decreased to original levels (7.0-7.7).

Experiment 2

An additional experiment was established because the soil used in the first

experiment may have initially had levels of P (3 g/kg) and other nutrients that were too low to support AMF fungi. The soil used in this experiment was a Chernozemic loam from the Winnipeg, MB region that was autoclaved (121°C for 90 minutes) and diluted with coarse sand (1 loam:3 sand). This resulted in a soil with 9 g/kg of P (see Table 5 for other nutrients). Hoagland's solution (1/5 strength, without P or micronutrients) was added 19 days after seeding, and every 2 weeks subsequently. The remainder of Experiment 2 was methodologically similar to Experiment 1. Nutrient levels of the soils used in Experiments 1 and 2 were determined by Norwest Laboratories (Winnipeg, Mb.). These soils will be subsequently referred to as "potting-soil" to distinguish them from "soil-inoculum" (see next section for explanation of soil-inoculum).

3. Inoculation with AMF

a) Rationale

There are several possible methods for the inoculation of experimental plants with AMF. Spores collected from "pot-cultures" (greenhouse-grown plants colonized by AMF) can be mixed with autoclaved soil, or can be pipetted directly onto roots of seedlings (Menge and Timmer, 1982). Alternatively, soil collected from the field can be mixed into autoclaved soil or placed as a discrete layer in pots. Arbuscular mycorrhizal fungal propagules in the soil are capable of colonizing experimental seedlings. The advantage of the "spore-method" is that the introduction of microorganisms other than AMF is minimized (Koide and Li, 1989); "soil-inoculum" includes many bacteria, fungi, protists, and invertebrates (*i.e.* nematodes) that may affect plant-growth. However, soil-inoculum has the advantage of reproducing more closely the biotic environment experienced by roots in the field (Smith and Smith, 1981). Effects of AMF

Table 5. Characteristics of soils used in experiments 1 and 2. All nutrient concentra6ions are in µg/g. NO_3-N was determined after $CaCl_2$ extraction, P and K after acetic fluoride extraction, Ca, Mg and Na after ammonium acetate extraction, and Cu, Fe, Mn,and Zn after DTPA extraction. Nand P were measured colorimetrically, and other elements by ICP spectroph otometry.

Experiment	pH	EC†	NO_3-N	P	K	Ca	Mg	Na	Cu	Fe	Mn	Zn	S	O.M.§ (%)
1	7.9	2.69	6.0	3	36	10700	310	458	1.8	66.6	4.7	2.7	191	0.6
2	7.8	0.6	20.0	9	133	988	246	8.9	0.7	31.1	8.5	0.8	19.0	0.9

†Electrical Conductivity (dS/m)

§Organic Matter

observed after the use of soil-inoculum, then, should more closely reflect effects in the field.

The experiments described in this, and following chapters depended on soil-inoculum for the introduction of AMF to experimental plants. The principal reason for this approach was the difficulty of establishing pot-cultures from spores isolated from salt pans in the Overflowing River region.

When designing experiments on the effects of AMF on plant growth, it is necessary to include a group of plants that are not colonized by AMF, but are grown under similar conditions as mycorrhizal plants. In the experiments described in this thesis, non-mycorrhizal plants were exposed to autoclaved (1 hr at 121°C) soil-inoculum. The "background-microflora" (*i.e.* microorganisms other than AMF) was partially reproduced in non-mycorrhizal plants using methods described in the next section.

b) Methodology

Soil-inoculum was collected from the same salt pan that was studied in Chapter II. On October 3, 1991 (Experiment 1) and on August 23, 1992 (Experiment 2), soil was removed from the root zone of *Agropyron trachycaulum*. Ten g of this soil (the "soil-inoculum") were added to each 10 cm pot as a narrow layer in the centre of the pot (the remainder of the pot was filled with autoclaved potting soil). Previous experiments had shown that plants inoculated in this way were well colonized after 4-6 weeks. An equal number of pots received autoclaved soil-inoculum.

The background microflora of untreated (*i.e.* non-autoclaved) soil-inocula was added to autoclaved soil-inocula using the following procedure: untreated soil (100 mL) was suspended in 1 L of water and poured through 500, 212, and 53 μm sieves. These sieves retain AMF spores and colonized roots, but allow the passage of bacterial and

non-mycorrhizal fungal propagules. Water and suspended materials that passed through the 53 μm sieve were collected, and mixed with the autoclaved soil-inoculum.

4. AMF-species in the inoculum

In numerous preparations of spore-extracts (see Appendix B for methodology) of soil from salt pans (or from "bait plants" exposed to this soil) in the Overflowing River area over a three year period (1991-1993) I have only found three species of AMF: *Glomus etunicatum* Beck.&Gerd, *G. macrocarpum* Tulasne & Tulasne, and *Entrophosphora infrequens* (Hall) Ames & Schneider. The soil-inoculum used in the experiments described in this chapter was taken from a site where *G. etunicatum* is consistently present (100-200 spores/100 g dry soil), *E. infrequens* is less common (0-7 spores/100 g dry soil), and *G. macrocarpum* has never been observed. Numerous attempts to infect plants (*Zea mays* L, *Hordeum jubatum* L., or *A. trachycaulum*) with *G. etunicatum* spores collected throughout the growing season from this site have all failed.

5. Seeding of pots and growth conditions

Seeds of *A. trachycaulum*, *Hordeum jubatum*, or *Puccinellia nuttalliana* were added to the pots. After seeding, plants were placed in a growth chamber with a cycle of 16 hours of light (22°C) and 8 hours of darkness (20°C). Light levels were maintained at 500 $\mu E.m^{-2}.sec^{-1}$ at the leaf tips throughout the experiment. The position of pots within the growth chamber was re-randomized every 2 weeks.

6. Salinity treatment

Each of 96 pots was thinned to 5 seedlings/pot 27 days after seeding. On day 37 salinity treatments began. Pots were randomly assigned to 4 salinity treatments (0, 6, 12, and 18 g/L NaCl). The salinity of irrigation water was gradually raised to final levels over a period of 11 days. Subsequently, each pot was watered with deionized water, and flushed with 100 mL of the appropriate concentration of saline water twice weekly. Care was taken to ensure that on other days the water-holding capacity of the soil was not exceeded, to avoid dilution of salts in the soil water.

7. Plant harvest

Plants were harvested 69 days after salt treatments began (105 days after seeding). Shoot and root biomass were determined, after the removal of a weighed subsample of the root system for quantification of AMF-colonization. The ratio of wet to dry root biomass was used to adjust root biomass for the loss of the subsample.

8. AMF-colonization

Root preservation and staining followed the method described in Appendix A. AMF-colonization was quantified using the grid-line intersect method (Kormanik and McGraw, 1982). Roots were spread out in a petri dish marked with a grid (1.3 cm interval). All intersections of roots with grid lines were observed with a stereomicroscope, and AMF-colonization at intersections was recorded. This allowed

the determination of percentage of root length colonized by AMF (PRLC), total root length (TRL), and total root length colonized by AMF (TCRL).

9. Shoot nutrient analysis

Dried shoots were ground in a Wiley mill, and digested in sulfuric acid and nitric acid (1:8) with added peroxide. Levels of Cu, Zn, and Na were measured by atomic absorption spectrophotometry, and P concentration was measured colorimetrically by the formation of the phosphate-molybdate complex (Murphy and Riley, 1962). These methods are described in detail in Appendix C.

Shoot nutrient concentrations and total levels are both presented, because each has distinct implications for plant growth. Nutrient concentration is relevant to the immediate physiological function of shoot tissue, whereas total levels are a measure of the total uptake of the nutrient, which may be important in future growth of the plant.

10. Data analysis

Effects of inoculation treatment and soil salinity on biomass, root to shoot ratio (RSR), nutrient concentration, total nutrient content, and TRL were analyzed by 2-way analysis of variance (ANOVA). Effects of soil salinity on PRLC and TCRL were analyzed by 1-way ANOVA, because levels of AMF-colonization in plants that received autoclaved inoculum were consistently very low. Consequently, plants exposed to autoclaved inoculum were excluded from the analysis. Biomass and tissue nutrient variables were log-transformed when necessary to meet ANOVA assumptions, and

percentage variables were arcsin-square root transformed before analysis (Ott, 1988). Individual differences among salinity treatments were determined to be significant if 95% confidence intervals did not overlap.

D.Results

1. Biomass

Results of 2-way ANOVA of shoot and root biomass and RSR are shown in Table 6, and means ± SE are displayed in Figure 12. In all three species, the 2-way ANOVA revealed that mycorrhizae (*i.e.* "untreated" inoculum) and salinity significantly reduced shoot biomass. Shoot biomass of non-mycorrhizal *P. nuttalliana* appeared to be relatively unaffected by salinity. The inhibitory effect of mycorrhizae was observed in all salinity treatments of *H. jubatum* and *P. nuttalliana*, but not of *A. trachycaulum*. Shoot biomass of mycorrhizal and non-mycorrhizal *A. trachycaulum* was similar at the 6 g/L NaCl level, which may account for the significant interaction between inoculum-treatment and salinity in this species.

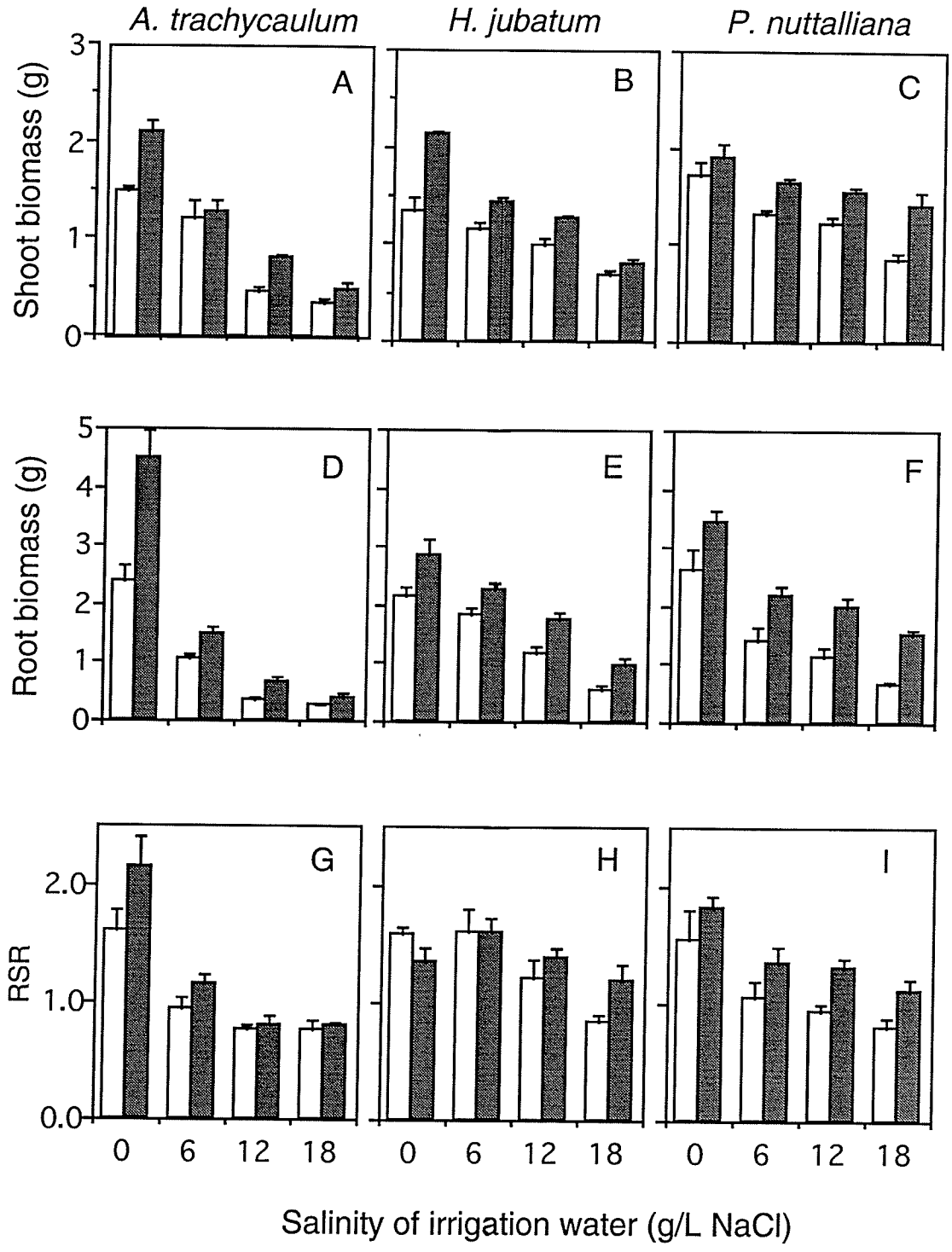
Root biomass of all 3 species also significantly declined with increasing salinity and in the presence of mycorrhizae. None of the interaction terms were significant. In all 3 species salinity led to a significant decrease in RSR. Mycorrhizal inoculation led to decreased RSR of *A. trachycaulum* and *P. nuttalliana*, but not of *H. jubatum*.

Table 6. Two-way ANOVA of the effect of inoculation and salinity treatments on shoot biomass, root biomass, and RSR of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana* (Experiment 1).

			F-ratio		
Host species	Source of variation	df	Shoot biomass	Root biomass	RSR
A. trachycaulum	Inoculation	1	20.7***	21.9***	6.5*
" "	Salinity	3	86.6***	83.5***	42.5***
" "	Inoculation x Salinity	3	3.39*	1.76	1.05
H. jubatum	Inoculation	1	37.4***	23.8***	0.64
" "	Salinity	3	58.4***	47.8***	8.61***
" "	Inoculation x Salinity	3	2.15	0.47	2.20
P. nuttalliana	Inoculation	1	27.0***	36.2***	11.4**
" "	Salinity	3	18.5***	34.3***	11.3***
" "	Inoculation x Salinity	3	1.45	0.014	0.051

*p<0.05; **p<0.01; ***p<0.001

Figure 12. Shoot biomass (A-C), root biomassw (D-F), and RSR (G-I) of mycorrhizal and non-mycorrhizal plants from Experiment 1. (A,D,G), *A. trachycaulum*; (B,E,H), *H. jubatum*; (C,F,I), *P. nuttalliana*; (*open square*), mycorrhizal; (*closed square*), non-mycorrhizal.


A. trachycaulum
H. jubatum
P. nuttalliana
A
B
C
D
E
F
G
H
I
Shoot biomass (g)
Root biomass (g)
RSR
0
1
2
3
4
5
0.0
1.0
2.0
0
6
12
18
Salinity of irrigation water (g/L NaCl)

2. Shoot nutrients

a) Phosphorus

Two-way ANOVA of shoot nutrient concentration (Table 7 Figure 13) showed that P concentration was similar in mycorrhizal and non-mycorrhizal *A. trachycaulum*, but was significantly higher in mycorrhizal *H. jubatum* and *P. nuttalliana*. Salinity increased P concentration in all 3 species, probably because of salinity-induced inhibition of shoot growth.

Results of 2-way ANOVA of total shoot nutrients are shown in Table 8, with means ± SE displayed in Figure 14. Total P of *A. trachycaulum* and *H. jubatum* was significantly lower in mycorrhizal plants, and total P of *A. trachycaulum* declined in the 12 and 18 g/L NaCl treatments. Total P was unaffected by mycorrhizae or salinity in the other species.

b) Copper

The only significant difference in copper concentration in shoot tissue was among salinity treatments of *A. trachycaulum*. The concentration of Cu increased in this species with increased salinity. Total Cu of all three species was significantly lower in mycorrhizal plants, and decreased as salinity increased. In *H. jubatum* there was a significant interaction between salinity and inoculum-treatment. This interaction was probably caused by a discrepancy in the effects of AMF on total Cu between the 6 g/L NaCl treatment and the 0, 12, and 18 g/L NaCl treatments. However, residuals of this analysis were not normally distributed, so caution in the interpretation of these results is recommended.

Table 7. Two-way ANOVA of the effect of inoculation and salinity treatments on the concentration of P, Cu, and Na in shoots of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana*..

			F-ratio		
Host species	Source of variation	df	P	Cu	Na
A. trachycaulum	Inoculation	1	0.033	0.42	0.55
" "	Salinity	3	35.3***	27.6***	27.7***
	Inoculation x Salinity	3	1.25	1.85	0.49
H. jubatum	Inoculation	1	6.28*	4.14	5.96*
" "	Salinity	3	32.4**	1.03	4.18*
	Inoculation x Salinity	3	0.82	1.67	8.10***
P. nuttalliana	Inoculation	1	8.90**	1.91	0.54
" "	Salinity	3	22.5***	1.04	1.08
	Inoculation x Salinity	3	2.66	0.96	1.13

*p<0.05
**p<0.01
***p<0.001

Figure 13. Concentration of P (A-C), Cu (D-F), and Na (G-I) in shoot tissue of mycorrhizal and non-mycorrhizal plants from Experiment 1. (A,D,G), *A. trachycaulum*; (B,E,H), *H. jubatum*; (C,F,I), *P. nuttalliana*; (*open square*), mycorrhizal; (*closed square*), non-mycorrhizal.

61b

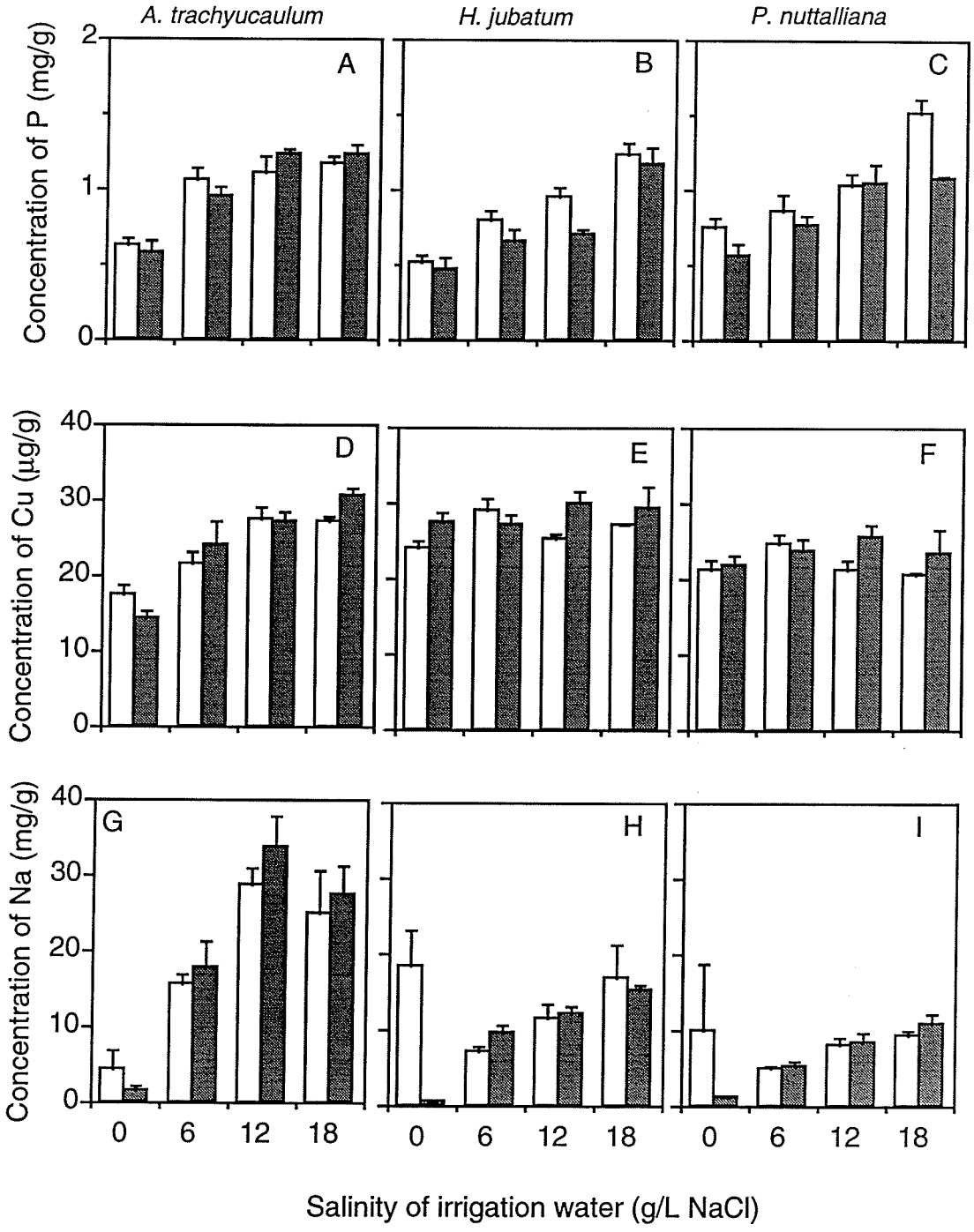

A. trachyucaulum
H. jubatum
P. nuttalliana
A
B
C
D
E
F
G
H
I
Concentration of P (mg/g)
Concentration of Cu (µg/g)
Concentration of Na (mg/g)
0
1
2
10
20
30
40
0
6
12
18
Salinity of irrigation water (g/L NaCl)

Table 8. Two-way ANOVA of the effect of inoculation and salinity treatments on the total amounts of P, Cu, and Na in shoots of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana*..

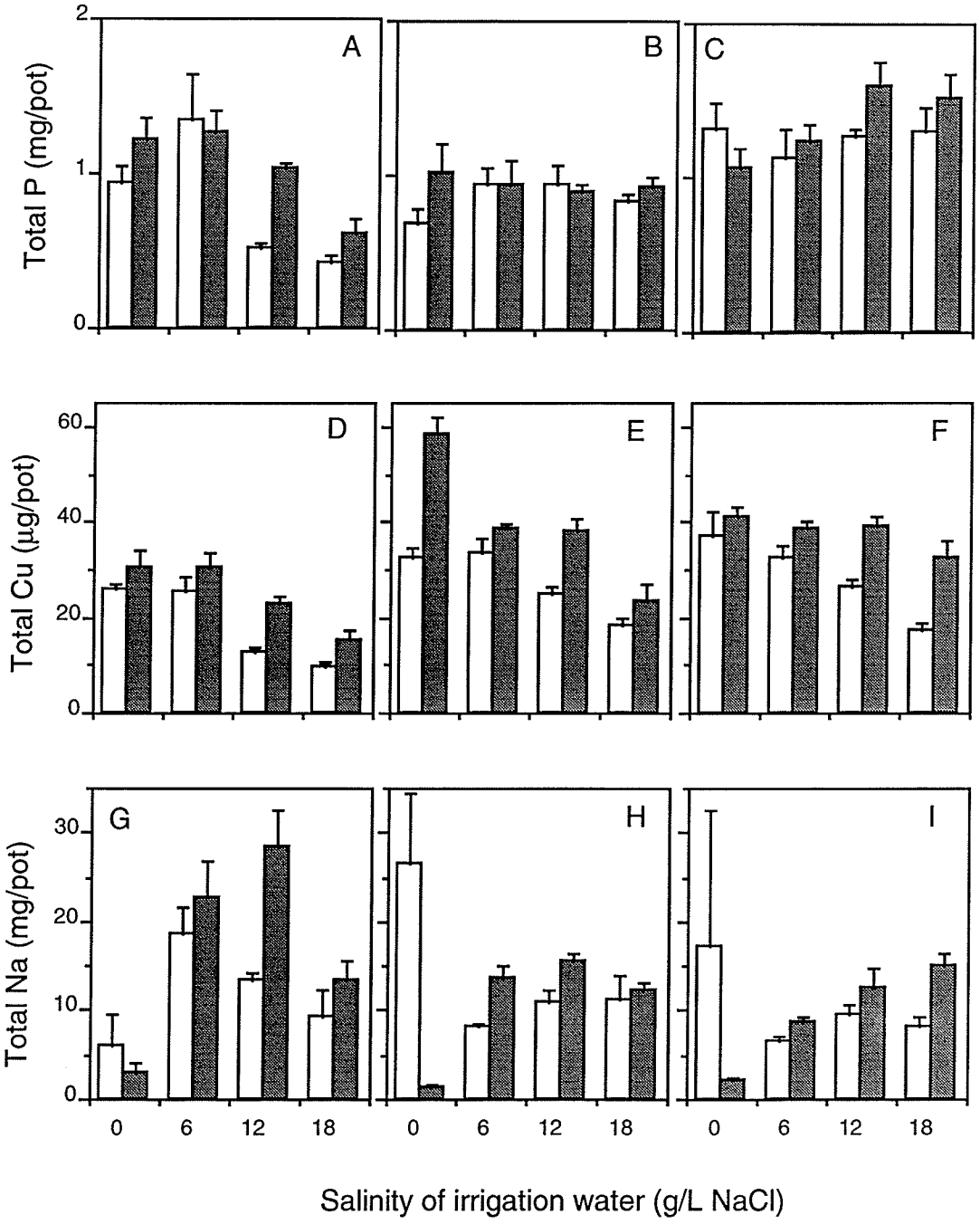
			F-ratio		
Host species	Source of variation	df	P	Cu	Na
A. trachycaulum	Inoculation	1	5.51*	16.3***	5.27*
" "	Salinity	3	12.4***	24.6***	13.2***
" "	Inoculation x Salinity	3	1.53	0.53	2.97
H. jubatum	Inoculation	1	4.36*	53.1***	2.42
" "	Salinity	3	0.26	35.7***	0.38
" "	Inoculation x Salinity	3	1.12	8.61***	10.7***
P. nuttalliana	Inoculation	1	1.09	23.5***	0.037
" "	Salinity	3	1.78	9.5***	0.206
" "	Inoculation x Salinity	3	1.61	1.86	1.58

*p<0.05

**p<0.01

***p<0.001

Figure 14. Total amounts of P (A-C), Cu (D-F), and Na (G-I) in shoot tissue of mycorrhizal and non-mycorrhizal plants from Experiment 1. (A,D,G), *A. trachycaulum*; (B,E,H), *H. jubatum*; (C,F,I), *P. nuttalliana*; (*open square*), mycorrhizal; (*closed square*), non-mycorrhizal.


A
B
C
D
E
F
G
H
I
Total P (mg/pot)
Total Cu (µg/pot)
Total Na (mg/pot)
0
1
2
20
40
60
10
30
0
6
12
18
Salinity of irrigation water (g/L NaCl)

c) Sodium

The concentration of Na in shoots of *A. trachycaulum* increased dramatically with salinity, and was similar in both mycorrhizal and non-mycorrhizal plants. Residuals from the 2-way ANOVA of *H. jubatum* and *P. nuttalliana* were not normally distributed. In both species Na concentration appeared to increase with salinity, although the increase was less marked than in *A. trachycaulum.* In plants of *H. jubatum* irrigated with non-saline water, shoot Na concentration appeared to be higher in mycorrhizal plants than in non-mycorrhizal plants.

The analysis of total shoot Na in *H. jubatum* and *P. nuttalliana* was also marred by non-normal distribution of residuals. There was a highly significant interaction between inoculum-treatment and salinity in *H. jubatum*, which appeared to be caused by the large increase in total Na in mycorrhizal plants in the non-saline treatment. This increase was also observed in *P. nuttalliana*, but the interaction term was not significant. There was a large amount of variation within the mycorrhizal non-saline treatment combination for both species. In contrast, mycorrhizal plants of *A. trachycaulum* in the non-saline treatment had similar total Na levels as non-mycorrhizal plants. Total Na in *A. trachycaulum* was significantly lower in mycorrhizal plants, but this effect was most apparent in the 12 g/L NaCl treatment.

3. AMF colonization and root length

The proportion of root length colonized (PRLC) of *A. jubatum* was significantly larger in the 6 g/L NaCl treatment than in the 12 and 18 g/L NaCl treatments (Table 9). PRLC was unaffected by salinity in the other species. In general, PRLC was lower in *P. nuttalliana* than in *H. jubatum* or *A. trachycaulum.* TCRL of *A. trachycaulum* was similar in the 0 and 6 g/L NaCl treatment, and decreased in the 12 and 18 g/L

Table 9. Effects of salinity on PRLC and TCRL of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana*. Plants that received autoclaved inoculum are not included. Means (± SE) indicated by different letters are significantly different.

Host species	Level of salinity[1]	PRLC (%)	TCRL (m)	n
A. trachycaulum	0	27.6 ab ± 6.2	13.5 ab ± 2.9	4
" "	6	54.6 a ± 3.7	12.0 a ± 1.8	4
" "	12	25.5 b ± 9.7	4.8 b ± 1.4	4
" "	18	22.7 b ± 3.9	4.2 b ± 1.5	4
H. jubatum	0	35.4 ± 2.2	22.8 a ± 2.4	3
" "	6	32.4 ± 3.0	12.5 b ± 2.6	3
" "	12	34.1 ± 4.8	8.1 bc ± 1.1	4
" "	18	19.7 ± 6.2	2.6 c ± 23.0	4
P. nuttalliana	0	6.9 ± 2.3	4.9 ± 1.8	4
" "	6	26.2 ± 14.9	10.9 ± 6.3	2
" "	12	16.6 ± 3.9	6.1 ± 1.4	4
" "	18	6.4 ± 1.4	2.6 ± 0.7	4

[1]g/L NaCl in irrigation water

treatments. In *H. jubatum* TCRL decreased with increased salinity, and TCRL of *P. nuttalliana* was not significantly affected by soil salinity.

Total root length (TRL) of *A. trachycaulum* was highly variable in the non-saline treatment, but not in the saline treatments (Figure 15), which makes interpretation of the analysis of TRL in this species problematic. Two-way ANOVA (Table 10) revealed strong effects of both mycorrhizae and salinity on TRL in *A. trachycaulum* and *H. jubatum*. In *P. nuttalliana*, however, the effect of salinity on TRL was smaller (F-ratio: 4.77) than in the other two species (F-ratios: 21.8 -*A. trachycaulum*; 21.4 -*H. jubatum*).

4. Experiment 2: shoot biomass

Two-way ANOVA of biomass (Table 11) revealed significant negative effects of mycorrhizae and salinity on shoot and root biomass of *A. trachycaulum* and *H. jubatum* (Figure 16). Salinity led to decreased shoot and root biomass of *P. nuttalliana*, but mycorrhizae had no effect. Salinity led to significantly increased RSR of *A. trachycaulum* and *H. jubatum*, and to decreased RSR in *P. nuttalliana*.

E. Discussion

1. Effects of AMF on host biomass

I observed an inhibition of shoot and root growth of mycorrhizal plants of three halophytic grasses. In Experiment 1 this effect was similar in non-saline and in saline soil. In Experiment 2, mycorrhizal inhibition of growth of *H. jubatum* was apparent only in the non-saline treatment, and growth of *P. nuttalliana* was unaffected by mycorrhizae. This suggests that under severe P-stress (Experiment 1) AMF inhibit

Figure 15. Total root length of mycorrhizal and non-mycorrhizal plants from Experiment 1. (A), *A. trachycaulum*; (B), *H. jubatum*; (C), *P. nuttalliana*; (*open square*), mycorrhizal; (*closed square*), non-mycorrhizal.

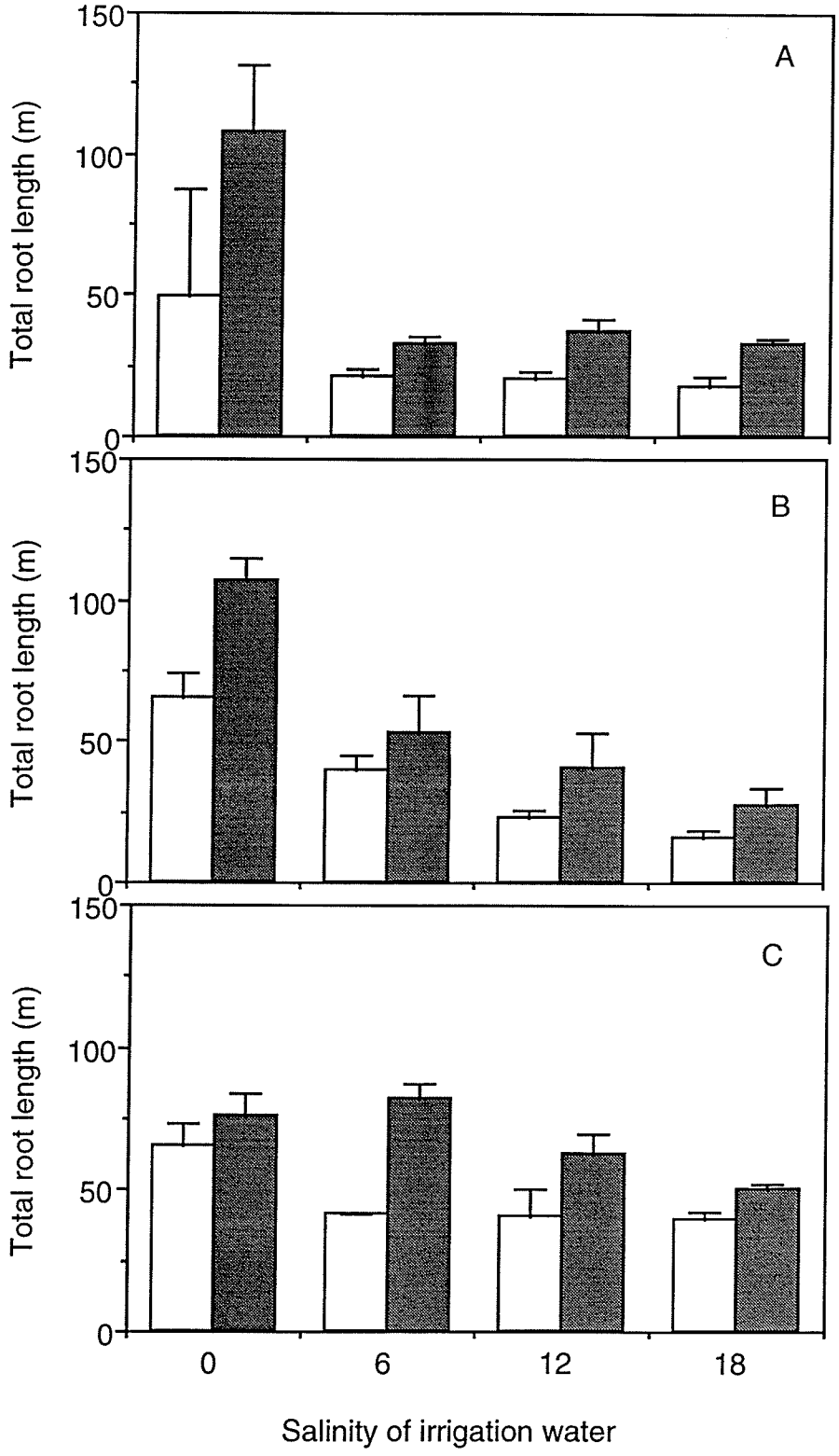

150
100
50
0
A
Total root length (m)
B
C
0
6
12
18
Salinity of irrigation water

Table10. Two-way ANOVA of the effect of inoculation and salinity treatments on the total root length of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana*..

Host species	Source of variation	df	F-ratio
A. trachycaulum	Inoculation	1	22.8***
" "	Salinity	3	21.8***
" "	Inoculation x Salinity	3	4.50*
H. jubatum	Inoculation	1	11.4**
" "	Salinity	3	21.4***
" "	Inoculation x Salinity	3	1.24
P. nuttalliana	Inoculation	1	14.7**
" "	Salinity	3	4.77*
" "	Inoculation x Salinity	3	1.67

*$p<0.05$

**$p<0.01$

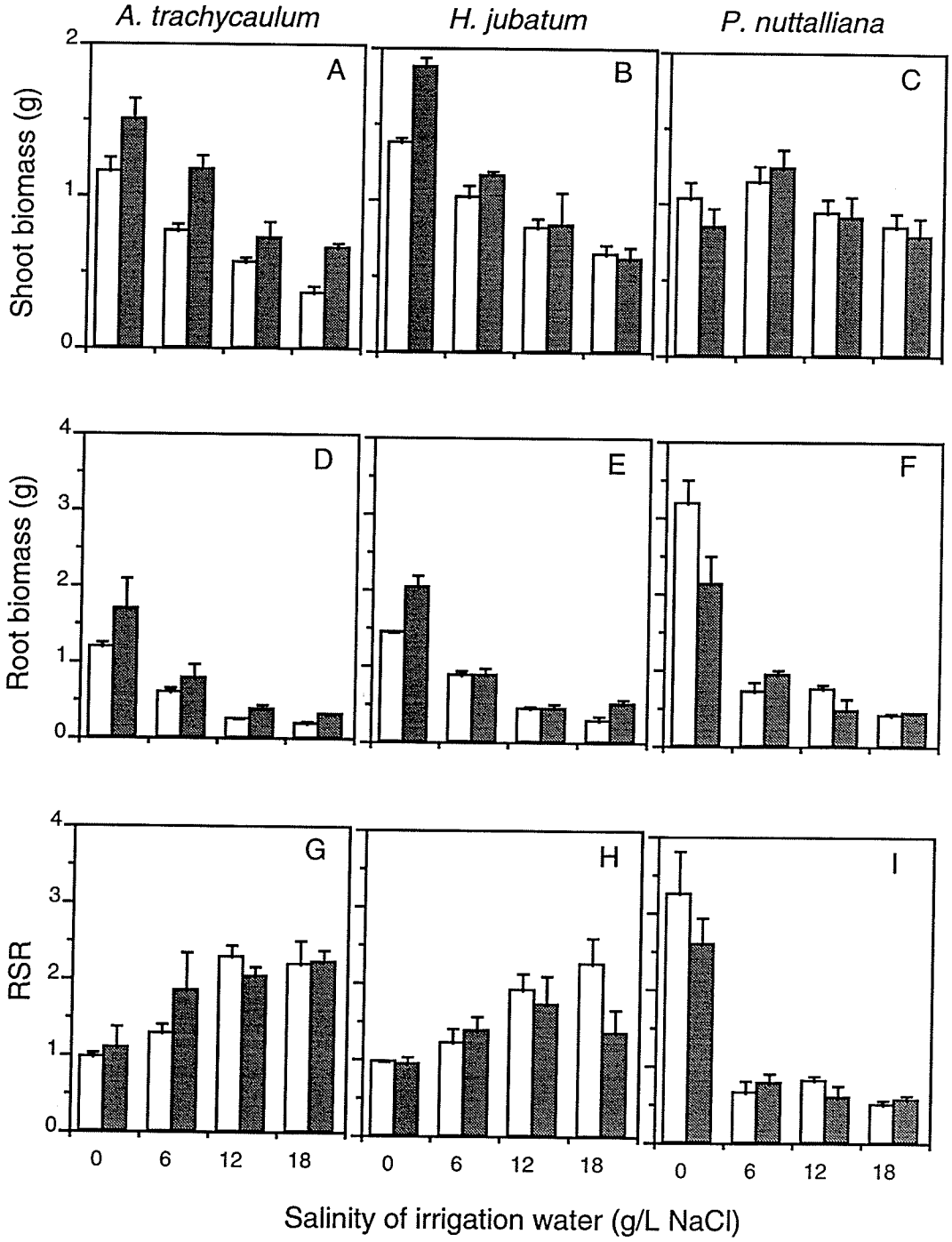
***$p<0.001$

Table 11. Two-way ANOVA of the effect of inoculation and salinity treatments on shoot biomass, root biomass, and RSR of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana* (Experiment 2).

			F-ratio		
Host species	Source of variation	df	Shoot biomass	Root biomass	RSR
A. trachycaulum	Inoculation	1	24.7***	5.59*	0.40
" "	Salinity	3	35.7***	36.8***	9.34***
" "	Inoculation x Salinity	3	0.77	0.45	0.88
H. jubatum	Inoculation	1	4.90*	5.89*§	1.98
" "	Salinity	3	38.9***	73.1***§	6.44**
" "	Inoculation x Salinity	3	3.15*	2.62§	1.98
P. nuttalliana	Inoculation	1	0.31	1.57§	0.31
" "	Salinity	3	3.73*	40.1***§	27.7***
" "	Inoculation x Salinity	3	0.50	2.86§	1.36

*p<0.05
***p<0.001
§log-transformed before analysis

Figure 16. Shoot biomass (A-C), root biomass (D-F), and RSR (G-I) of mycorrhizal and non-mycorrhizal plants from Experiment 2. (A,D,G), *A. trachycaulum*; (B,E,H), *H. jubatum*; (C,F,I), *P. nuttalliana*; (*open square*), mycorrhizal; (*closed square*), non-mycorrhizal.


A. trachycaulum
H. jubatum
P. nuttalliana
A
B
C
D
E
F
G
H
I
Shoot biomass (g)
Root biomass (g)
RSR
0
1
2
3
4
0
6
12
18
Salinity of irrigation water (g/L NaCl)

growth in saline and non-saline conditions, but under moderate P-stress (Experiment 2) AMF may not adversely affect plants in saline soil. In Experiment 1 the level of P may have been too low to support adequate transfer of P from the fungus to the plant. Perhaps the acquisition of P via mycorrhizae in plants that have extensive, fibrous root systems is relatively expensive (in terms of carbon) in extremely P-deficient soils. It should be pointed out that there are important differences between the environmental conditions of the experiments described here, and field conditions; in field soils there is usually a well-developed network of AMF-hyphae (Read, 1992). This network was virtually absent in my experimental pots, although a network presumably developed over the course of the experiment. The disruption of the hyphal network may have decreased the ability of AMF to colonize newly formed roots, and transfer P to them (Evans and Miller, 1990).

Another major difference between the soils used in experiments 1 and 2 was that calcium (Ca) was present in much higher concentrations in Experiment 1. This may have inhibited AMF-hyphal growth and nutrient uptake in the soil in Experiment 1. Soil Ca-content similar to that in Experiment 1 has been shown to inhibit mycorrhizal development in maize (Gryndler *et al.*, 1991).

Pond *et al.* (1984), Duke *et al.*, (1988), and Poss *et al.* (1988) all observed growth-stimulation of hosts by AMF in saline soil. However, all of these studies used host plants that typically react favorably to AMF (onion, tomato, and citrus). Plants used in our study were C_3 grasses (Guy *et al.*, 1986), which often do not respond positively to AMF (Hetrick *et al.*, 1988a, 1990). Temperate C_3 grasses typically have extensive, fibrous root systems that may not benefit from mycorrhizal colonization. My observations of negative, or neutral effects of mycorrhizae on host growth may be typical of C_3 halophytic grasses.

It is also possible that the negative effects of AMF on plant growth were caused by characteristics of the population of *G. etunicatum* that was present in the soil-inoculum. Arbuscular mycorrhizal fungi vary in their ability to promote the growth of their hosts; AMF that produce large numbers of spores may be less beneficial to their hosts than AMF that produce few spores (Johnson *et al* 1992).

The possibility that pathogenic bacteria or fungi were present in the soil inoculum cannot be discounted. However, since non-mycorrhizal fungi were not observed in the roots, and AMF were able to colonize roots in all salinity treatments, it is probable that the reduction of plant biomass by untreated inoculum was driven by AMF.

2. Effects of salinity on RSR

In experiment 1, increased salinity led to decreased RSR in all 3 species, whereas in experiment 2 it led to increased RSR in *A. trachycaulum* and *H. jubatum*, suggesting that root growth is particularly sensitive to the combined stress of low nutrients and salinity that was present in experiment 1. Another interpretation is that P-deficiency in experiment 1 led to the allocation of the majority of plant resources to root growth. In saline soil, however, the inhibition of shoot growth resulted in a lowered P-demand, that resulted in decreased allocation of resources to roots.

The ratio of root biomass to shoot biomass of *P. nuttalliana* was greatest in non-saline soil in both experiments, perhaps because of a greater requirement for nutrients in this species when its growth is not limited by salinity.

3. Effects of AMF on host nutrition

a) Phosphorus

The concentration of P in shoots of all 3 species increased with salinity, suggesting that P-uptake was less inhibited by soil salinity than was leaf expansion. This has been

observed in crop plants grown in sand or solution culture, but not in experiments using soil (Grattan and Grieve, 1992). P-uptake is usually suppressed in plants growing in saline soil, partly because of reduced activity of phosphate in the soil solution (Sharpley *et al.*, 1992). In our study, all of the plants showed symptoms of severe P-deficiency that were alleviated by the addition of $NaH_2PO_4.2H_2O$ on day 69. Much of the shoot phosphate may have been taken up after this addition. In this respect, our findings are similar to studies of crop plants in saline solution culture. The extensive root system of both mycorrhizal and non-mycorrhizal plants in our study may have allowed the plants to take advantage of the P-application and absorb substantial amounts of P.

Mycorrhizal and non-mycorrhizal *H. jubatum* and *P. nuttalliana* accumulated similar amounts of total P, despite the significantly lower biomass of mycorrhizal plants. Furthermore, both species had higher concentrations of shoot P in mycorrhizal than in non-mycorrhizal plants. One interpretation of these results is that decreased shoot biomass of mycorrhizal plants was partially compensated for by improved P-uptake. However, in these two species salinity did not have an affect on total P accumulation, either. An alternative interpretation, then, is that biomass reductions, whether they are caused by AMF or salinity, do not affect the total P accumulation of *H. jubatum* or *P. nuttalliana*. Interestingly, the least salt-tolerant species (*A. trachycaulum*) had decreased levels of total P at the highest salinity levels. Perhaps the ability of halophytes to maintain P-uptake rates over a range of salinities is important to the survival of halophytes in saline soil.

b) Copper

The concentration of Cu in shoots of *H. jubatum* and *P. nuttalliana* was not affected by salinity or AMF. Increased soil salinity led to increased Cu concentration in the

shoots of *A. trachycaulum*. This may have been caused by a salinity-induced reduction in the specificity of ion uptake in *A. trachycaulum*.

d) Sodium

Increased soil salinity led to increased concentrations and total amounts of Na in all three species. The large amount of Na accumulated supports the idea that Na uptake, in combination with reduced water content and accumulation of organic solutes, allows halophytic grasses to continue water uptake in saline soil (Glenn, 1987). However, it is also apparent that decreased salt-tolerance is linked with increased Na concentration in shoot tissue. The concentration of Na in shoots was higher in *A. trachycaulum* than in *H. jubatum*, which had a higher concentration than *P. nuttalliana*. This hierarchy mirrors the relative salt-tolerance of the three species in the field (Burchill and Kenkel, 1991).

In non-saline soil, the concentration and total amounts of Na in mycorrhizal plants were higher than in non-mycorrhizal plants, although this effect was less evident in *A. trachycaulum*. The AMF present in the inoculum (*G. etunicatum*) may accumulate Na in non-saline, resulting in transfer of large amounts of Na to host plants.

4. Effects of increased salinity on root growth and AMF colonization

The proportion of root length colonized (PRLC) and TCRL of *A. trachycaulum* were similar in the 0 and 6 g/L NaCl treatments. This may have been caused by a relatively greater impact of salinity on growth of roots of *A. trachycaulum* as compared with growth of mycorrhizal hyphae. Alternatively, moderate concentrations of NaCl

may stimulate mycorrhizal colonization; a similar effect has been observed with magnesium (Gryndler *et al.*, 1991).

In all three species, AMF-colonization was not inhibited by soil salinity. This may be a reflection of the ability of AMF to grow in saline soil, or it may reflect the ability of AMF to maintain colonization within roots when soil salinity inhibits hyphal growth in the soil.

PRLC was lowest in *P. nuttalliana*, which suggests that this species is able to maintain AMF-colonization at a lower level. This may explain the absence of mycorrhizal inhibition of growth of *P. nuttalliana* in Experiment 2. The low levels of AMF-colonization observed in this species may lead to decreased host growth in highly P-deficient soil (Experiment 1), but not in moderately P-deficient soil (Experiment 2).

Total root length was affected by AMF and salinity in a similar pattern as was root biomass, with some exceptions. For example, low salinity (6 g/L NaCl) led to a precipitous drop in the TRL of non-mycorrhizal *A. trachycaulum*, but TRL was unaffected in non-mycorrhizal *P. nuttalliana* at that level of salinity. Since root biomass of *P. nuttalliana* decreased in the low salinity treatment, there may have been a change in root architecture (*e.g.* decreased root diameter and more frequent branching) that partially compensated for the decrease in biomass. Such architectural changes may be an important mechanism of salt tolerance in halophytes.

There are several important conclusions to be made from this chapter. It is evident that plant allocation of resources to roots (RSR) is strongly affected by both soil salinity and soil nutrient levels. In this respect, and in others (*e.g.* total P accumulation, PRLC, effects of mycorrhizae in experiment 2) the response of the strongly salt-tolerant *P. nuttalliana* was different from the response of the weakly salt-tolerant *A. trachycaulum.* Therefore, there may be fundamental differences between halophytes and glycophytes,

not only in their response to salinity, but also in their response to AMF, their resource requirements and their response to varying levels of soil nutrients.

Arbuscular mycorrhizal fungi led to decreased biomass of all three species, at all levels of soil salinity. Mycorrhizal plants growing in non-saline soil had higher total amounts of Na in shoot tissue than non-mycorrhizal plants. The effect of AMF on shoot phosphorus was complex, and inconsistent among the three species. Mycorrhizal *H. jubatum* had similar total amounts of shoot P as non-mycorrhizal plants, whereas mycorrhizal *A. trachycaulum* had lower aounts shoot P than non-mycorrhizal plants.

Chapter V

Variation in response of *Hordeum jubatum* L. to arbuscular mycorrhizal fungi introduced via soil inoculum

A. Abstract

The objective of this chapter is to determine the extent of variation among AMF (introduced via soil-inoculum) of their effects on growth of *Hordeum jubatum* L. Greenhouse-grown plants were exposed to AMF in soil inoculum collected from 8 sources (from 4 sites, within a 5 km radius). The experimental design allowed the analysis of effects of each combination of 3 factors: (*i*) inoculum source; (*ii*)inoculum treatment (untreated *vs* autoclaved); (*iii*) soil salinity (0 *vs* 12 g/L NaCl).

Only 3 of the inoculum sources resulted in >5% colonization of root length by AMF. AMF in these 3 sources led to decreased plant biomass in non-saline soil. Infection of roots by *Pythium* spp. probably contributed to the adverse effects of untreated inoculum.

The major potential source of variation in effects of AMF in these salt pans appears to be variation in inoculum potential. Infection by *Pythium* and other root pathogens may also lead to variation in effects of soil inocula on plant growth.

2. Introduction

As discussed previously (Chapter I), the prevalence of AMF in most undisturbed terrestrial environments has led to the belief that they may be important in

the function of natural systems. Consequently there have been efforts to examine the effects of AMF on their hosts in the field (Fitter, 1985), or in experimental environments that are as close as possible to the field environment. One way to more closely approach field conditions is to use soil-inoculum collected from the field. It is then certain that the density of AMF-propagules is similar to that found in the field; this is not necessarily the case if greenhouse-grown pot-cultures are the source of inoculum. The use of soil-inoculum also allows for the action of microorganisms in field soil that interfere with, or stimulate AMF-colonization (Bagyaraj and Menge 1978, Meyer and Linderman 1986, Hetrick *et al* 1988b). Although this may interfere with the direct comparison of mycorrhizal to non-mycorrhizal plants, it gives a more realistic, and ecologically meaningful assessment of the effects of AMF in field systems.

However, the use of field soil to inoculate plants with AMF may adversely affect the reproducibility of experimental results, because of variation in AMF inoculum-potential, and confounding effects of non-mycorrhizal microorganisms. Both of these factors should result in variation among soil samples in the effects of AMF on plant growth. It is worthwhile to assess the extent of this variation, because of the common use of soil inoculum in studies of AMF (*e.g.* Pond *et al.* 1984, Cerligione *et al.* 1988, Hetrick *et al.* 1994).

The problem of confounding microorganisms in soil inoculum can be partially addressed by the addition of soil filtrates (Chapter IV, section C,3), but this is clearly not an optimal solution, because microorganisms adhering to large soil particles or roots would not be present in filtrates, but may have effects on AMF-colonization or plant growth.

Another potential problem with the use of soil-inoculum is that its use requires the assumption that the AMF-species present in the soil-inoculum are species that are locally important. However, substantial local variation in the species-composition of

AMF has been observed (Koske and Halvorson, 1981; Sylvia, 1986).

Host response to AMF varies among AMF-species (Mosse, 1972; Jensen, 1982; Schubert and Hayman, 1986), and among populations of AMF (*e.g.* Stahl *et al.* 1990; Stahl and Christensen, 1991). The effects of AMF introduced via soil inoculum, then, may not be representative of the array of AMF-species present at a particular site. It should be emphasized that the use of spore-inoculum in mycorrhizal studies, as recommended by Koide and Li (1989), does not overcome this problem. Indeed, the use of spores derived from a pot-culture of a single AMF-species is likely to exacerbate the problem. Soil inoculum is useful in mycorrhizal studies because it integrates the effects of the AMF-species present, and the effects of non-mycorrhizal microorganisms. The real problem, then, is not the use of soil inoculum, but lies in the potential within-site and between-site variation in the contribution of AMF to observed effects on plant growth. A study of within-site variation in plant response to AMF has demonstrated that this variation may be substantial (Henkel *et al* 1989).

Chapter IV established that AMF colonization of *H. jubatum*, *P. nuttalliana*, or *Agropyron trachycaulum* resulted in decreased plant growth at a range of soil salinities. However, it is unknown if this reflects the range of potential effects of AMF collected from the salt pans in the Overflowing River region.

The objective of this chapter is to compare the response of *H. jubatum* to AMF in 8 soil samples collected from 4 salt pans. This chapter also begins to examine the nature and extent of local variation in AMF.

C. Materials and Methods

1. Collection of soil-inocula

Eight soil samples (surface 20 cm), representing eight "inoculum sources" were taken from 4 salt pans within a 5 km radius (Figure 17) in June, 1992. Pans #3 and #4 are directly adjacent to the shore of Lake Winnipegosis, and are connected by saline meadows. Pan #3 was the site of the studies described in Chapters II and III (with the exception of the experimental transplant of *P. nuttalliana* seedlings to a Hordeum zone, which took place in pan #4). The soil inoculum used in Chapter V was collected from the Agropyron zone of pan #3. Pans #1 and #2 are inland, and each is isolated from other salt pans by boreal forest (predominantly *Picea glauca* and *Populus tremuloides*).

Soil samples were taken in June, 1992 from the root zones of 5 different plant species (Table 12), each of which commonly dominates vegetation zones at the salt pans (Burchill and Kenkel, 1991). Soil samples were stored at 4°C and were used to inoculate plants within 2 weeks.

2. Potting soil

The potting soil was a 1:1 mixture of Chernozemic loam (Winnipeg region) and coarse sand, and had the following characteristics after autoclaving: pH (7.8); organic matter (2.3%); nitrate (96 μg/g); phosphate (30 μg/g); potassium (363 μg/g); sulphate (23 μg/g); calcium (2500 μg/g); magnesium (616 μg/g); sodium (23 μg/g); copper (0.8 μg/g); iron (11.1 μg/g); manganese (9.8 μg/g); zinc (0.9 μg/g). Nutrient analysis was conducted by Norwest Laboratories (Winnipeg, MB).

Figure 17. Location of salt pans. Eight soil samples were taken from four salt pans (labelled 1-4) near the northwest shore of Overflow Bay Lake Winnipegosis. 1 cm = 560 m.

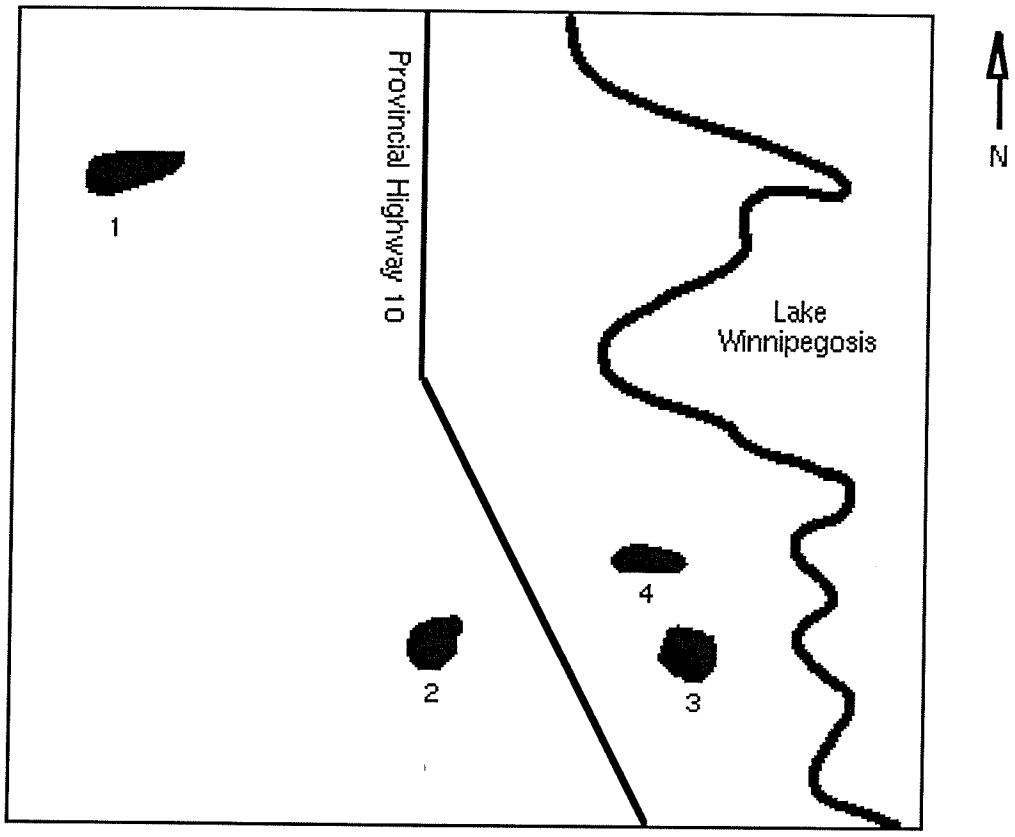

Provincial Highway 10
Lake
Winnipegosis
N
1
2
3
4

TABLE 12. Sources of AMF-inoculum.

Soil	Nearby plant[1]	Site
A	*Hordeum jubatum*	1
B	*Distichlis stricta*	1
C	*Hordeum jubatum*	2
D	*Hordeum jubatum*	3
E	*Agropyron trachycaulum*	3
F	*Spartina gracilis*	3
G	*Hordeum jubatum*	5
H	*Calamagrostis inexpansa*	5

[1]Inocula were taken from vegetation zones dominated by the stated plant.

3. AMF inoculation and experimental design

As in Chapter IV, soil inoculum was added as a narrow layer in the centre of each pot. Soil from each inoculum source was added to 20 pots (10 received untreated inoculum, and 10 received autoclaved (121°C for 1 hr) inoculum). Each group of ten pots was further divided into two groups of 5 pots; one group was always watered with deionized water (non-saline treatment) and the other was flushed twice-weekly with saline water (12 g/L NaCl). Therefore, the experiment consisted of a factorial experiment with 3 factors: (A) Inoculum source (8 levels); (B) Inoculum treatment (2 levels); (C) Salinity treatment (2 levels). There were 5 replicates of each combination of these factors.

4. Background microflora

Using the method described in Chapter IV, filtrates of untreated soil were added to pots that had autoclaved inoculum. Plants that were exposed to autoclaved inoculum from a particular inoculum source received filtrates from the same inoculum source. In this experiment, filtrates were not mixed into autoclaved inoculum, but were pipetted directly into the soil around seedlings 14 days after seeding.

5. Seeding of pots and growth conditions

Seeds of *H. jubatum* collected from site 3 were added to each pot. After 21 days, pots were thinned to 5 seedlings/pot. Plants were maintained in a greenhouse with 130 $\mu E.m^2.sec^{-1}$ supplemental fluorescent lighting (measured at the leaf tips). Lights were approximately 30 cm above leaf tips throughout the experiment. Pots were watered daily with deionized water, and salinity treatments began 35 days after seeding.

Plants were harvested 83 days after seeding. The number of tillers per pot was recorded, and a subsample of the root system (0.3 g wet weight) was preserved in 50%

ethanol for microscopy. Shoot, root, and inflorescence biomass were measured after drying at 70°C.

6. Root microscopy

Roots were cleared and stained following the method described in Appendix A. AMF-colonization, arbuscules, and vesicles were quantified using the method of McGonigle *et al.* (1990). Roots were arrayed horizontally on a slide and examined in a compound microscope (250x). Vertical passes were made through the roots, and at each intersection of the eyepiece-crosshair and a root, the presence of mycorrhizal hyphae, arbuscules, or vesicles was noted. I also recorded the presence of non-mycorrhizal fungi. One hundred and eighty intersections were recorded for each root system.

7. Data analysis

Shoot and root biomass, the ratio of root biomass to shoot biomass (RSR), number of tillers, inflorescence number, and inflorescence biomass were analyzed by 3-way analysis of variance (ANOVA), with three fixed factors: (*i*) inoculum source; (*ii*) inoculum treatment, and (*iii*) salinity. There were significant interaction terms in all variables except RSR and inflorescence biomass; therefore, attention will be focused on trends within the data set that explain interactions. Soil source was considered to be a fixed factor, because soil inocula were collected semi-randomly; they were taken randomly within specifically chosen vegetation zones at specific sites (salt pans). Therefore, the analysis of inoculum source is interpreted only in respect to the vegetation zones that were sampled, and not to the salt pans as a whole.

The percentage of root length containing structures of AMF and other fungi was calculated for each pot. Results are presented without analysis, because of extreme heterogeneity of variance among factor combinations.

D. Results

1. Biomass

a) Shoot biomass

Three-way ANOVA revealed significant main effects of inoculum source and salinity treatment, a significant interaction betweeen inoculum source and inoculum treatment, and a significant interaction among inoculum source, inoculum treatment, and salinity treatment (Table 13). Four patterns in response to inoculation and salinity treatments are apparent (Figure 18) among the 8 inoculum sources: (*i*) shoot biomass was higher in plants receiving autoclaved inocula than in plants receiving untreated inocula, regardless of soil salinity (sources A, B, and F); (*ii*) shoot biomass was higher in plants receiving autoclaved inocula than in plants receiving untreated inocula, but only in the non-saline treatment (source E); (*iii*) shoot biomass was higher in plants receiving untreated inocula than in plants receiving autoclaved inocula, but only in the non-saline treatment (sources D and G); (*iv*) shoot biomass was similar in plants receiving autoclaved or untreated inocula (soils C and H).

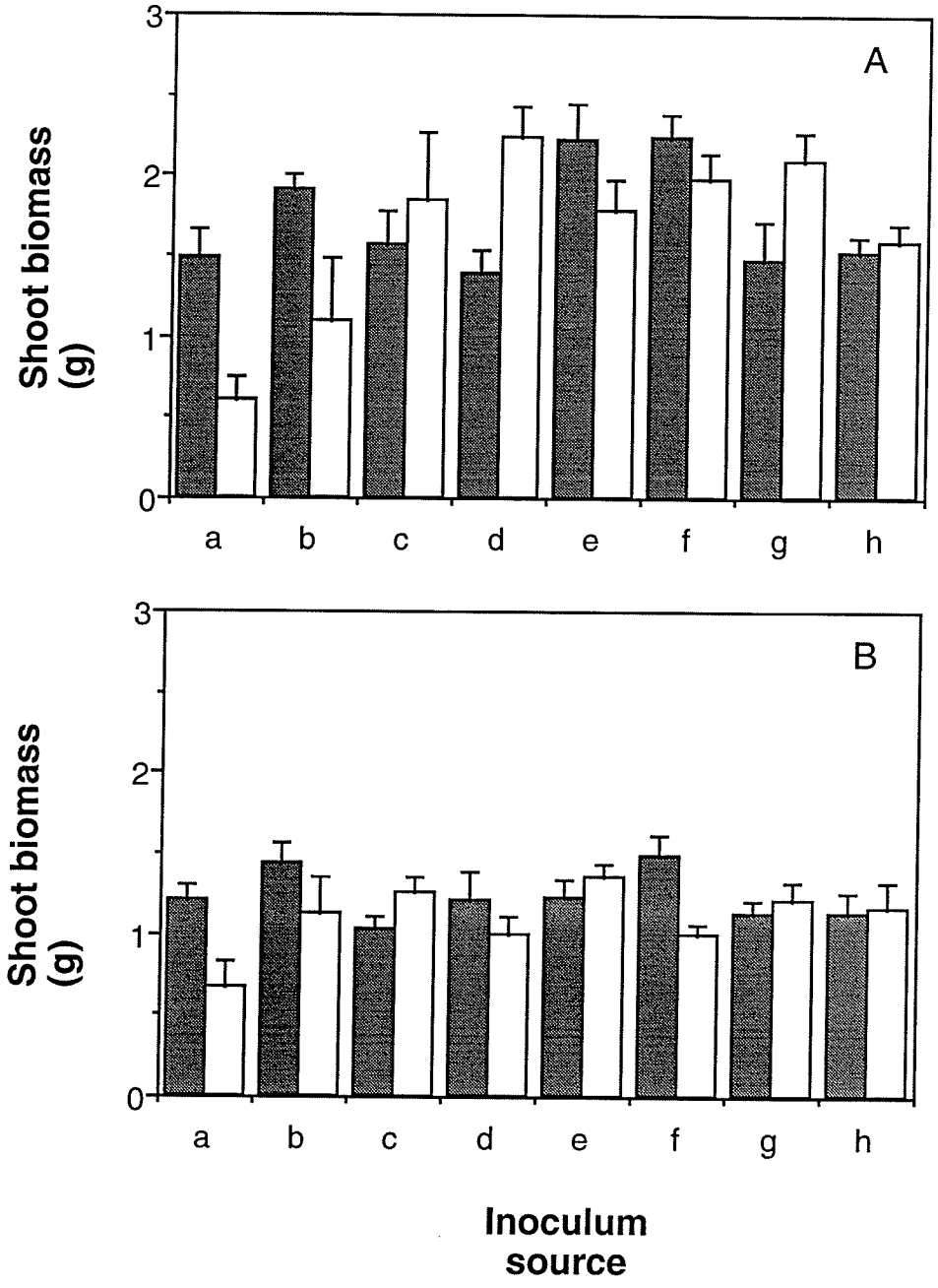
The significant 3-way interaction implies that the effect of salinity was not consistent in all combinations of inoculum source and inoculum treatment. This interaction is illustrated by the disparity in response to salinity in plants inoculated with inoculum sources E and H. For inoculum source E, the effects of soil salinity were much more severe in pots with untreated inoculum than in pots with autoclaved inoculum, whereas shoot biomass in pots receiving untreated or autoclaved inocula from inoculum source H were similarly affected by salinity.

Table 13. F-ratios from ANOVA of biomass, tillers, and inflorescences of *H. jubatum*.

Source of Variation[a]	df	Shoot biomass	Root biomass	RSR[b]	No. of tillers	No. of INF[c]	INF[c] biomass
SOIL	7	5.5***	1.7	1.7	2.0	2.4*	3.0**
INOC	1	2.7	9.5**	5.4*	0.4	1.1	4.8*
SALT	1	67.9***	8.2**	16.8***	26.6***	5.1*	19.4***
SOIL x INOC	7	5.2***	5.2***	1.8	3.3**	2.2*	1.8
SOIL x SALT	7	2.1	1.1	0.6	2.0	1.3	1.6
INOC x SALT	1	0.3	1.0	2.4	0.4	2.1	0.3
SOIL x INOC x SALT	7	2.4*	1.4	1.5	0.8	0.9	0.8

[a]Abbreviations: SOIL: inoculum source; INOC: -inoculation treatment; SALT: salinity treatment.
[b]Root-to-shoot ratio
[c]Inflorescence
*p <0.05
**p < 0.01
***p <0.001

Figure 18. Shoot biomass of *Hordeum jubatum*. Soil inoculum from eight sources was autoclaved (*shaded bars*), or untreated (*unshaded bars*). (A), non-saline soil; (B), saline soil.


A
B
Shoot biomass (g)
Shoot biomass (g)
0
1
2
3
a
b
c
d
e
f
g
h
Inoculum source

b) Root biomass

Root biomass was significantly affected by inoculum treatment and salinity treatment (Table 13). There was a significant interaction between inoculum source and inoculum treatment. The pattern of response to the 3 experimental factors was similar to that of shoot biomass, except that there was a greater reduction of root biomass, as compared with shoots, of plants given untreated inoculum from sources A or B (Figure 19). The response of roots to inoculation with inoculum source G was also different from that of shoots; root biomass of plants in non-saline soil was not greater in untreated inoculum as compared with autoclaved inoculum.

c) Root-to-shoot ratio

The analysis of RSR helps to clarify the relationship of root responses to shoot responses. The main effects of inoculation treatment and salinity treatment were significant, and none of the interaction terms were significant (Table 13). Untreated inoculum generally led to decreased RSR, as compared with autoclaved inoculum (Table 14). This effect was particularly strong in soil sources A and B.

In contrast, RSR increased in most cases in saline soil, indicating that soil salinity had a greater effect on shoot growth than on root growth. This is consistent with the results of Chapter V.

2. Tiller number

Soil salinity significantly reduced tiller number, and there was a significant interaction between inoculum source and inoculation treatment (Tables 13 and 14). In general, the effects of inoculum source and treatment on tiller number were similar but less strong than effects on shoot biomass. For example, there was more than a two-fold difference in mean shoot biomass of plants receiving autoclaved *vs* untreated "A"

Figure 19. Root biomass of *Hordeum jubatum*. Soil inoculum from eight sources was autoclaved (*shaded bars*), or untreated (*unshaded bars*). (A), non-saline soil; (B), saline soil.

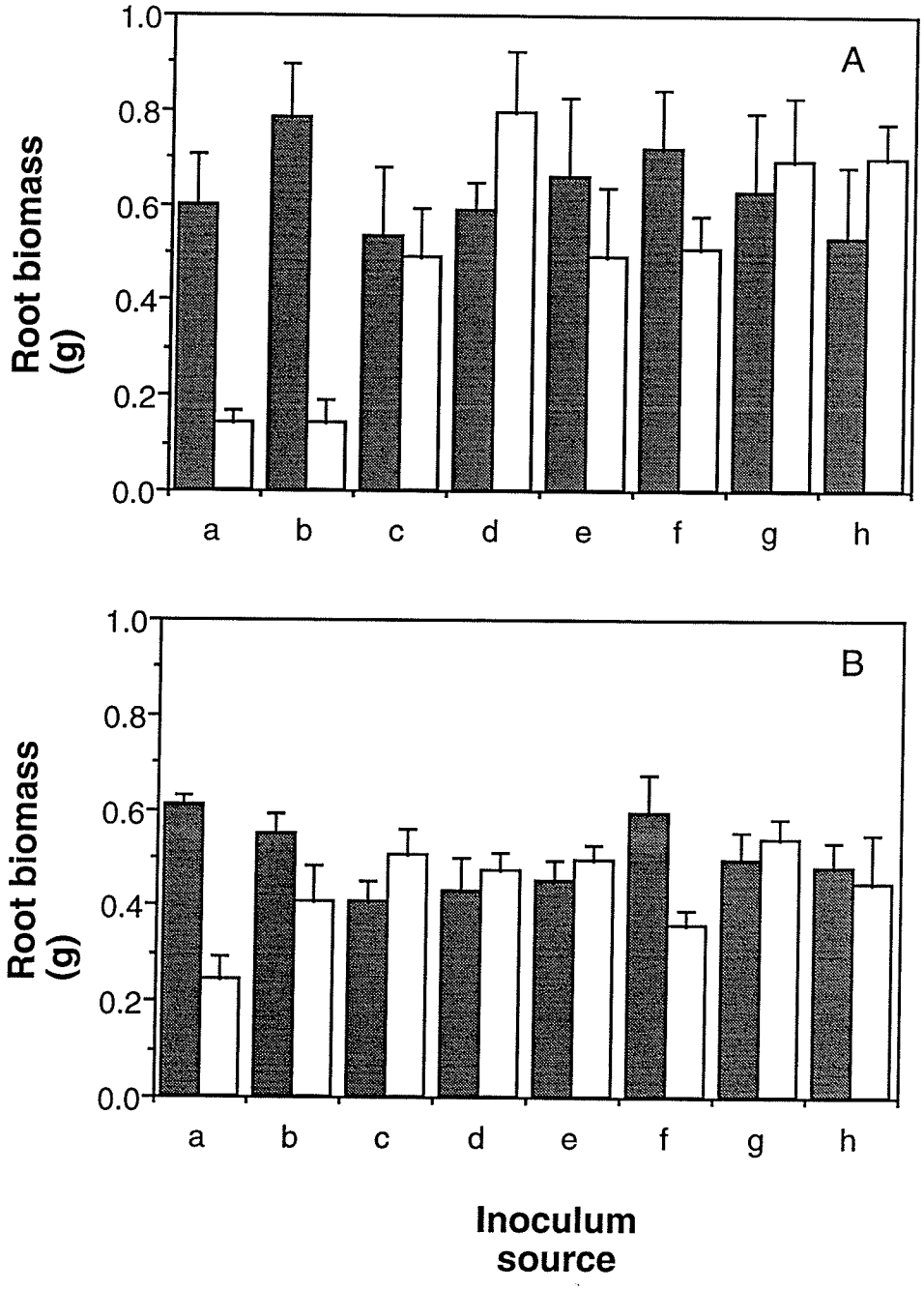

1.0
0.8
0.6
0.4
0.2
0.0
Root biomass (g)
A
a
b
c
d
e
f
g
h
B
Inoculum source

Table 14. Vegetative characteristics of *H. jubatum*. (A) Plants were irrigated with non-saline water; (B) plants were irrigated with saline (12 g/L NaCl) water.

(A) Non-saline

Inoculum source	Inoculum treatment	RSR[ab]	Tillers per pot[b]	INF[c] per pot[b]	INF[c] biomass (mg/pot)[b]
A	autoclaved	0.40 ± 0.03	23 ± 2	2.6 ± 0.5	86 ± 25
B	" "	0.41 ± 0.05	30 ± 2	2.4 ± 0.5	110 ± 26
C	" "	0.33 ± 0.07	23 ± 3	2.0 ± 0.8	103 ± 30
D	" "	0.42 ± 0.02	26 ± 2	1.2 ± 0.4	76 ± 26
E	" "	0.31 ± 0.09	28 ± 1	3.2 ± 1.0	142 ± 47
F	" "	0.33 ± 0.06	31 ± 2	4.2 ± 1.0	205 ± 46
G	autoclaved	0.40 ± 0.06	25 ± 2	2.4 ± 0.7	89 ± 30
H	" "	0.35 ± 0.10	26 ± 2	2.0 ± 0.3	74 ± 16
A	untreated	0.26 ± 0.05	19 ± 2	0.6 ± 0.4	15 ± 11
B	" "	0.15 ± 0.02	22 ± 3	2.4 ± 1.1	77 ± 50
C	" "	0.29 ± 0.07	28 ± 4	3.6 ± 0.9	149 ± 49
D	" "	0.36 ± 0.06	32 ± 3	2.8 ± 0.2	107 ± 12
E	" "	0.29 ± 0.08	30 ± 3	3.6 ± 0.9	102 ± 24
F	" "	0.27 ± 0.06	29 ± 2	2.8 ± 0.8	129 ± 46
G	" "	0.32 ± 0.04	34 ± 3	3.4 ± 1.3	122 ± 36
H	" "	0.44 ± 0.03	26 ± 3	1.6 ± 0.4	62 ± 8
(B) Saline					
A	autoclaved	0.52 ± 0.05	23 ± 1	2.0 ± 0.5	60 ± 22

B	" "	0.39 ± 0.03	26 ± 2	3.0 ± 0.9	105 ± 37
C	" "	0.40 ± 0.05	22 ± 2	1.6 ± 0.5	49 ± 15
D	" "	0.35 ± 0.02	21 ± 3	2.8 ± 0.7	90 ± 25
E	autoclaved	0.37 ± 0.03	22 ± 2	2.4 ± 0.7	75 ± 16
F	" "	0.40 ± 0.04	26 ± 4	2.6 ± 0.4	100 ± 4
G	" "	0.43 ± 0.03	21 ± 1	2.0 ± 0.6	62 ± 6
H	" "	0.43 ± 0.03	23 ± 2	2.0 ± 0.6	53 ± 17
A	untreated	0.39 ± 0.04	20 ± 2	0.2 ± 0.2	6 ± 6
B	" "	0.36 ± 0.02	24 ± 2	2.2 ± 0.8	62 ± 24
C	" "	0.40 ± 0.03	25 ± 1	1.4 ± 0.6	59 ± 24
D	" "	0.49 ± 0.05	20 ± 2	1.4 ± 0.4	33 ± 11
E	" "	0.37 ± 0.04	22 ± 2	3.4 ± 0.4	96 ± 19
F	" "	0.36 ± 0.02	23 ± 1	0.8 ± 0.4	25 ± 11
G	" "	0.45 ± 0.03	25 ± 2	1.8 ± 0.7	50 ± 23
H	" "	0.37 ± 0.03	24 ± 2	2.4 ± 0.7	64 ± 23

[a]Ratio of root biomass to shoot biomass.
[b]Mean ± SEM of 5 replicates.
[c]Inflorescence(s)

inoculum (non-saline treatment). The corresponding mean number of tillers was 23 (autoclaved) *vs* 19 (untreated).

3. Reproductive effort

a) Number of inflorescences

There were significant differences in inflorescence number due to inoculum source and salinity treatment (Table 13). The interaction between inoculation source and inoculation treatment was also significant. Differences among treatment combinations in inflorescence number were similar to shoot biomass differences, with several exceptions (Table 14). Plants exposed to untreated inoculum from sources A or B had less shoot biomass than plants with autoclaved inoculum. Untreated inoculum from source A also reduced inflorescence number, but untreated inoculum from source B did not affect inflorescence number. Similarly, untreated inoculum from source E (non-saline soil) did not reduce inflorescence number, despite reduced shoot biomass.

b) Inflorescence biomass

Three-way ANOVA revealed significant main effects of inoculum source, inoculum treatment, and soil salinity on inflorescence biomass. Interaction terms were not significant. There appeared to be more variation associated with inflorescence biomass than with shoot biomass. For example, the coefficient of variation (CV: sd/mean x 100) of inflorescence biomass in the autoclaved inoculum from source B (non-saline soil) was 52.9%, whereas the CV of shoot biomass in the same factor combination was 13.26%. Despite this difference in variation, patterns of inflorescence biomass were similar to patterns of shoot biomass.

4. Root colonization by fungi

a) Arbuscular-mycorrhizal fungi

High levels of AMF-colonization were observed in plants exposed to untreated inocula from sources A and B (Figure 20). Inoculum from other sources (except source E) led to AMF-colonization of less than 10% of root length. A similar pattern was evident in the proportion of root length with arbuscules.

b) Non-mycorrhizal hyphal fungi

Non-mycorrhizal hyphal fungi occupied less than 5% of root length in all factor combinations (Table 15). They were most common in plants in non-saline soil that received untreated inoculum from sources A or B.

c) Plasmodiophorales

Colonization of the root cortex by an unidentified plasmodiophorin was common in virtually all factor combinations. The appearance of meronts (single-nucleus stage preceding plasmodia formation) and plasmodia was similar to *Polymyxa graminis*, but sporangia were never observed. This organism was most common in roots of plants in saline soil that were exposed to untreated inoculum, regardless of the source of the inoculum (Table 15).

d) *Olpidium*

Zoosporangia and cysts characteristic of *Olpidium* spp. were common in plants non-saline soil with untreated inoculum from source B or G (Table 15). *Olpidium* was uncommon in other factor combinations.

Figure 20. AMF-colonization of roots of *H. jubatum* grown in non-saline soil (*shaded bars*) or non-saline soil (*unshaded bars*). Roots exposed to autoclaved inoculum are not included. (A), proportion of root length colonized by hyphae, arbuscules, or vesicles (PRLC); (B), proportion of root length with arbuscules (PRLA).

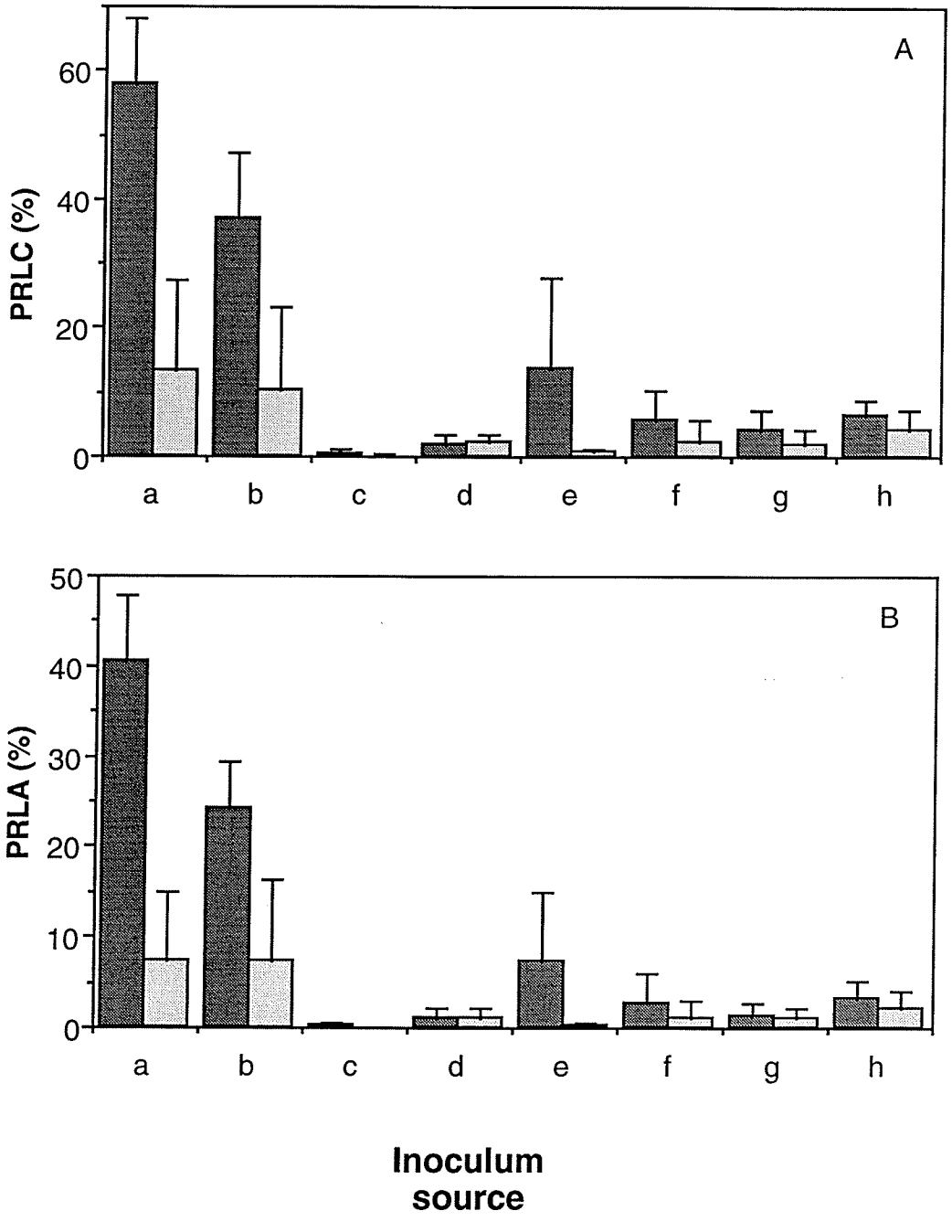

A
PRLC (%)
60
40
20
0
a
b
c
d
e
f
g
h
B
PRLA (%)
50
40
30
20
10
0
a
b
c
d
e
f
g
h
Inoculum source

Table 15. Non-mycorrhizal fungi in root tissue.(A) Plants were irrigated with non-saline water; (B) plants were irrigated with saline (12 g/L NaCl) water.

(A) Non-saline

Inoculum source	Inoculum treatment	n[a]	hyphal fungi[b]	Plasmodio-phorales[b]	Olpidium spp[b].	Pythium spp[b].
A	autoclaved	3	0	2.4 ± 1.8	0.2 ± 0.2	3.9 ± 2.6
B	" "	3	0	1.5 ± 1.1	0	0
C	" "	3	0.7 ± 0.6	2.0 ± 1.5	0.4 ± 0.4	5.7 ± 6.6
D	" "	5	0.4 ± 0.4	0.6 ± 0.5	1.2 ± 2.2	1.1 ± 1.7
E	" "	5	0.1 ± 0.2	1.0 ± 1.0	2.3 ± 4.2	5.1 ± 7.1
F	" "	5	0	1.6 ± 2.3	0	1.0 ± 1.6
G	autoclaved	4	0.1 ± 0.2	0.1 ± 0	0	2.9 ± 3.8
H	" "	5	0.1 ± 0.2	0.9 ± 1.0	0	1.8 ± 1.6
A	untreated	4	2.2 ± 1.2	0.6 ± 0.5	0.3 ± 0.4	11.8 ± 7.0
B	" "	3	2.8 ± 1.0	1.3 ± 0.6	17.4 ± 3.7	24.4 ± 11.3
C	" "	5	0	2.2 ± 3.1	1.0 ± 1.8	1.7 ± 2.1
D	" "	5	0.1 ± 0.2	0.4 ± 0.4	0	0.7 ± 0.7
E	" "	4	1.1 ± 1.0	4.4 ± 4.8	0.3 ± 0.2	3.6 ± 3.1
F	" "	4	0.4 ± 0.4	1.4 ± 0.5	2.2 ± 2.0	3.9 ± 3.4
G	" "	5	0.1 ± 0.2	5.2 ± 7.3	12.4 ± 12.1	4.9 ± 4.9
H	" "	3	0.6 ± 0.4	0	0	0

(B) Saline

A	autoclaved	4	0	2.1 ± 1.4	0.6 ± 0.6	0
B	" "	3	0	3.3 ± 1.3	0	0
C	" "	5	0.1 ± 0.2	7.1 ± 4.9	0	0
D	" "	5	0.1 ± 0.2	8.1 ± 6.0	0.3 ± 0.6	0
E	autoclaved	4	0	7.4 ± 6.4	0	0.3 ± 0.2
F	" "	5	0	11.2 ± 9.6	0	0
G	" "	4	0.1 ± 0.2	3.6 ± 2.0	0	4.7 ± 7.1
H	" "	5	0	5.2 ± 5.3	0	0
A	untreated	3	0.7 ± 0.6	11.5 ± 7.3	0.4 ± 0.4	0
B	" "	4	0	10.7 ± 6.1	1.5 ± 0.9	0
C	" "	5	0	22.7 ± 15.6	0	0
D	" "	5	0.1 ± 0.2	23.0 ± 4.5	0.2 ± 0.4	0
E	" "	3	0.2 ± 0.2	10.7 ± 5.7	0.6 ± 0.6	0
F	untreated	5	0	18.4 ± 8.1	0	0
G	" "	5	0	11.9 ± 12.6	2.6 ± 2.8	0
H	" "	5	0	10.7 ± 13.2	0	0

[a]number of replicates
[b]infection (% of root length)

e) *Pythium*

Gametangia, sporangia, and hyphae characteristic of *Pythium* spp. were most common in plants in non-saline soil with untreated inoculum from source A or B (Table 15). A low level of *Pythium* infection was consistently observed in plants that were watered with non-saline water and were exposed to autoclaved inoculum from any soil source.

E. Discussion

1. Effects of AMF on growth of H. jubatum

a) Growth enhancement

Untreated inoculum from sources D and G led to improved host growth as compared with autoclaved inoculum from the same sources, but this is unlikely to have been caused by AMF, because of the low level of AMF-colonization. The improved growth could be caused by microorganisms other than AMF that were present in untreated inoculum, and stimulated plant growth, or were present in autoclaved inoculum, and suppressed plant growth. These two possibilities cannot be distinguished, because autoclaved inoculum (with added "background microflora") and untreated inoculum from sources D and G led to similar levels of non-mycorrhizal fungi in roots. Other microorganisms that were not monitored in this study (*e.g.* bacteria), may have been responsible.

b) Growth reduction

AMF were at least partially responsible for growth reductions of plants exposed to untreated inoculum from source A and B. *Pythium* was also common in these plants, but only in the non-saline treatment. Since growth reductions were also observed in the

saline treatment, it is likely that AMF contributed to the poor growth of plants exposed to soil from source A or B.

The soil-inoculum used in both experiments, described in Chapter IV, was collected from source E of the current experiment. As was previously observed, untreated source E inoculum led to decreased shoot biomass in the current study, although to a much lesser extent than inoculum from sources A or B. Therefore, AMF collected from saline soil in the Overflowing River region consistently inhibit growth of *H. jubatum* by AMF. The inhibition in saline soil of AMF-colonization was not observed in the previous study, perhaps because of higher inoculum density. Inoculum used in the previous study was collected in August, which may be a time of peak numbers of mycorrhizal propagules.

Inspection of spores collected from sources A,B, and E revealed that *Glomus etunicatum* is virtually the only species of AMF present in soil from source E, and *Glomus macrocarpum* is the only species observed in soil from sources A and B. It therefore appears that both species have similar, adverse affects on the growth of *H. jubatum.*

The prime objective of this study was to assess variation in response of *H. jubatum* to AMF collected from different vegetation zones, and different salt pans in the Overflowing River region. Evidently, there is much variability in response, but the bulk of this variability may result from variation in the amount of AMF inoculum present in the soil. Inoculum from sources C,D,F,G and H led less than 5% PRLC. One explanation for these low levels is low density of mycorrhizal propagules in the soil-inoculum. Few spores were observed in bait plants exposed to soil from these sources, perhaps indicating that few spores were present in the experimental inoculum. Soil-inoculum from source D collected in April led to well colonized root systems (Chapter VI); therefore it appears that there may be substantial temporal variation in propagule

density in soils in the Overflowing River salt pans. Since temporal variation in propagule density has been observed in other habitats (Scheltema *et al.*, 1987) the timing of collection of soil-inoculum may well be important.

It is also possible that other factors in soil inoculum from sources C,D,F,G, and H inhibited AMF-colonization. This inhibition could have been caused by bacteria or fungi (Hetrick *et al.*, 1988b).

2. Pathogenic microorganisms

a) General comparison of untreated and autoclaved inoculum

Roots of plants that received autoclaved or untreated inocula were often colonized by non-mycorrhizal fungi that may have contributed to the complexity of plant responses. The introduction of "background microflora" to plants with autoclaved inocula was partially successful. Non-mycorrhizal fungi present in roots exposed to untreated inocula were also present in roots exposed to autoclaved inocula; unfortunately, the level of infection was very different (for example, examine the proportion of root length infected by *Pythium* and *Olpidium* in roots inoculated with soil from sources A,B, or G that was autoclaved, as compared with untreated soil). This experiment illustrates the need for mycorrhizal researchers to examine experimental roots (from both "mycorrhizal" and "non-mycorrhizal" plants) at high magnification, to allow the detection of pathogenic fungi.

b) *Pythium*

Pythium is an opportunistic plant pathogen that can cause extensive damage to plants, although this is usually most common and severe in seedlings. *Pythium* was common in plants in non-saline soil that received untreated inoculum from source A or B. This fungus may have been responsible for the observed inhibition of plant growth,

since this was the only group of plants that had greater that 10 % infection of root length by *Pythium*.

It is also possible that combined action of *Pythium* and AMF contributed to the severe inhibition of root growth. Certainly, AMF-colonization does not appear to have protected *H. jubatum* from infection by *Pythium* in our study. AMF-induced protection of plants from pathogens such as *Pythium* may be dependent on extensive colonization of roots by AMF prior to pathogen attack (Azcón-Aguilar and Barea, 1992). In the current experiment roots were probably attacked by *Pythium* and AMF at the same time, which may have prevented AMF from protecting roots, especially if the basis of AMF-induced plant protection is preemption of space within roots.

c) *Olpidium*, Plasmodiophorins and hyphal fungi

It is difficult to ascribe growth responses of *H. jubatum* to non-mycorrhizal hyphal fungi, *Olpidium*, or Plasmodiophorins. These fungi rarely infected a large proportion of the root system, and little root damage from infection was evident (unlike *Pythium*, which caused extensive breakdown of root structure). Interestingly, Plasmodiophorins were present in all experimental groups, and were particularly common in roots of saline plants exposed to untreated inoculum from *any* source. Although some aspects of the Plasmodiophorales have been well studied (*e.g.* taxonomy and cytology -Karling, 1968), little is known of their ecology in natural or cultivated systems. The transmission of viruses from Plasmodiophorins may also have contributed to growth suppressions.

Untreated inoculum from source G led to greater shoot biomass than autoclaved inoculum, and roots of plants exposed to untreated inoculum had substantial infections of *Olpidium* (>12% of root length infected). Controlled experiments would be required to determine if there was a causal relationship between these two observations.

3. Interactions between soil salinity and root infection

Apart from plasmodiophorins (all inoculum sources) and AMF (sources A and B), there was very little fungal colonization of roots of plants irrigated with saline water. Furthermore, plants exposed to saline water and untreated A or B inoculum were similar in size to non-saline plants with the same inoculum. In this case, soil salinity did not decrease plant growth, because of salinity-induced inhibition of deleterious microorganisms. Although one would hesitate to advocate soil salinization as a method of controlling plant pathogens, it seems worthwhile to examine the impact of fluctuations in soil salinity on pathogen populations in natural systems.

4. Conclusions

It is clear that there is a great potential for inter-inoculum variation in the effects of AMF on plant growth. The chief source of this variation appears to be variation in inoculum density, although non-mycorrhizal microorganisms are also important. There is a need for more knowledge on the importance and extent of pathogenic soil microorganisms in natural systems, and their interactions with indigenous mycorrhizae.

Within-site and between-site variation in AMF-effects on plant growth increases the complexity of the question: What is the importance of AMF in ecological processes in natural systems? Further studies of this variation, and its sources, is needed to help address this important question.

Chapter VI

Effects of arbuscular mycorrhizal fungi on competition between grasses of inland salt pans

A. Abstract

The aim of this greenhouse experiment was to determine the effects of arbuscular mycorrhizal fungi (AMF) on competition between *Agropyron trachycaulum*, *Hordeum jubatum* and *Puccinellia nuttalliana*, grasses that are common in salt pans of north-central Manitoba. AMF were inoculated onto roots via soil inocula collected from two sites (sites 1 and 3 of Chapter V).

AMF suppressed the growth of *A. trachycaulum*, and AMF from one of the sites (site 1) suppressed the growth of *H. jubatum*. *P. nuttalliana* was unaffected by AMF. The overall intensity of competition was increased by AMF from site 1, but not from site 3. Inocula from the two sites also had distinct effects on the outcome of competition between *A. trachycaulum* and *H. jubatum*. Autoclaved (with "background" microflora restored) and untreated inoculum from site 1 led to suppression of *A. trachycaulum* by *H. jubatum*.

Growth of *P. nuttalliana*, the most salt-tolerant species, was strongly inhibited by *A. trachycaulum* or *H. jubatum*. This was not alleviated by AMF, despite the AMF-driven suppression of growth of *A. trachycaulum* and *H. jubatum*.

B. Introduction

On a theoretical basis, interspecific competition should be particularly common and intense among plants because of the similarities in resource requirements among

plants (Grubb, 1977); all plants require the same array of mineral nutrients, and virtually all plants depend upon light as a source of energy for growth and reproduction. The extravagant diversity of plant taxons and growth-forms in most communities is initially surprising in view of this similarity in resource requirements. A variety of theories have been proposed to explain this problem (*e.g.* Grime, 1979; Tilman, 1982; Aarssen, 1983). A central facet of all of these theories is that plant species vary in their "competitive ability", a concept that describes a plant's ability to tolerate the presence of interspecific competitors, as well as its ability to suppress interspecific competitors (Goldberg, 1990).

The basis of a plant's superior competitive ability is often related to growth rate; *i.e.* plants that grow faster are able to preempt soil space and deprive slower-growing plants of light and other resources (Grime, 1977).

Many plants appear to be highly dependent on mycorrhizal fungi for seedling establishment and subsequent vegetative growth (Mosse *et al.*, 1981). Therefore, mycorrhizae should be an important part of the overall suite of characteristics that make up a plant's competitive ability. It then follows that the presence or absence of mycorrhizae in field soils will have important repercussions on the competitive ability of plants that are dependent on mycorrhizae. Mycorrhizal fungi would also be expected to affect the competitive ability of plants that grow less well when colonized by mycorrhizae (*e.g.* Buwalda and Goh, 1982; Anderson and Liberta, 1989; Hetrick *et al* 1990).

Several studies have confirmed that AMF can have dramatic effects on the outcome of interspecific competition. For example, Fitter (1977) observed that the superior competitive ability of *Holcus lanatus* over *Lolium perenne* was dependent on the presence of AMF, despite the lack of a strong growth response to AMF in monoculture.

Plants that respond strongly to AMF in monoculture also appear to respond to AMF when competing with other plants. The grass *Andropogon gerardii*, which is highly dependent on AMF, is only able to outcompete the grass *Koeleria pyramidata* when AMF are present (Hetrick *et al.* 1989, 1994).

Another reason for recent interest in AMF involvement in competition is the observation that two or more individual plants can be interconnected through a common mycorrhizal network (Newman 1988; Eissenstat and Newman 1990). Such hyphal links could conceivably decrease the intensity of competition, by allowing the flow of nutrients from competitively superior to inferior plants. Competition intensity could also be increased, if nutrients flow from the inferior to the superior competitor. Several studies have in fact documented the suppression of competitively dominant plants, allowing increased growth of subordinate plants (Allen and Allen, 1984, Grime *et al* 1987). This suppression, however, could be caused by other mechanisms than the transfer of nutrients from dominant to subordinate plants; it could also be caused by the formation of ineffective symbioses in dominant plants that result in loss of carbon from the plant, without the benefit of improved mineral nutrition from the mycorrhiza.

Previous studies of AMF and plant competition (*e.g.* Crowell and Boerner 1988, Hetrick *et al.* 1989, Koide and Li 1991, Hartnett *et al.* 1993, Hetrick *et al.* 1994) have generally focused on competition between plants that differ substantially in their response to AMF. However, in some communities the majority of competitive interactions may involve species that are often well-colonized by AMF, but are not dependent on AMF for growth and reproduction. For example, the plants studied in Chapter IV (*Agropyron trachycaulum*, *Hordeum jubatum*, and *Puccinellia nuttalliana*) do not appear to be dependent on AMF for growth. They typically occur in discrete vegetation zones in the salt pans. *P. nuttalliana* and *H. jubatum* are rarely observed in the Agropyron zone (Burchill and Kenkel, 1991) despite their evident ability to grow in

soil similar to that found in the Agropyron zone (Jones, 1991). They may be prevented from establishment in the Agropyron zone because of their inferior competitive ability; this may then result in the relegation of *H. jubatum* and *P. nuttalliana* to more saline soil, a "refuge" from superior competitors (Kenkel *et al* 1991).

The aim of this chapter is to discover if AMF affect the intensity of competition, and if the outcome of pair-wise competition between *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana* changes when AMF are present.

I hypothesize that AMF will decrease the intensity of intraspecific and interspecific competition, since all of the competing species display reduced growth when colonized by AMF (Chapter IV). I also hypothesize that AMF will affect the outcome of interspecific competition between *P. nuttalliana* and the other species, because of the decreased colonization of *P. nuttalliana* by AMF (Chapter IV), and the absence of growth suppression of *P. nuttalliana* by AMF (Chapter IV, experiment 2).

As in Chapters IV and V, AMF were introduced via field-collected soil-inoculum. The experiment was duplicated to allow the comparison of inocula collected from site 1 (soil A in Chapter V) and site 3 (soil D in Chapter V).

C. Materials and Methods

1. AMF-Inoculum collection

Soil inoculum was collected from Hordeum zones at sites 1 and 3 on April 21, 1993. This collection time was chosen so that AMF-inoculum levels would reflect levels in the field at the time of spring regrowth. Soil was stored at 4°C until use (June 24, 1993). In the experiment described in Chapter V, soil inoculum from the Hordeum zone of site 3 stimulated growth of *H. jubatum*. This stimulation was probably not due to AMF, because of low levels of AMF colonization in roots. Soil inoculum from site 1 led to severe inhibition of root growth, possibly through the combined action of AMF and

Pythium spp. Spores of *Glomus etunicatum* are very common at site 1, but are rare at site 3, where *Glomus macrocarpum* is common.

2. Experimental design

This greenhouse experiment incorporated combinations of two factors: inoculum treatment (autoclaved *vs* untreated), and pot composition. There were 4 categories of pot composition for each of three plant species (species "A" in the following example): (*i*) three individuals of species A; (*ii*) six individuals of species A; (*iii*) three individuals of species A and three of species B; (*iv*) three individuals of species A, and three of species C. Therefore, in the complete experiment there were six monoculture treatments (two for each species), and three mixture treatments that tested competition between *A. trachycaulum* and *H. jubatum*, between *A. trachycaulum* and *P. nuttalliana*, and between *H. jubatum* and *P. nuttalliana*. The experiment, then, contained nine distinct categories of pot composition, and there were four different pot-composition categories for each species.

The inclusion of the treatment with six conspecific individuals allowed comparison of the effects of intraspecific and interspecific competition (the effect of competition between three individuals and three conspecific individuals can be compared to the effect of competition between three individuals and three individuals of another species).

Each combination of inoculation treatment and pot-composition category was replicated 6 times, and the experiment was duplicated to allow comparison of inocula from the 2 sites. This gave a total of 108 pots inoculated with soil from each site (9 pot-composition categories x 2 inoculation treatments x 6 replicates), and a total of 216 pots in the complete study.

3. Growth conditions

The experimental soil was a steamed Chernozemic loam from the Winnipeg region, mixed 1:1:1 with coarse sand and peat moss. The resulting potting soil had the following characteristics: pH, 6.5; organic matter, 4.6%; nitrate, 8.0 μg/g; phosphate, 27 μg/g; potassium, 318 μg/g; calcium, 2.26 mg/g; magnesium, 638 μg/g; sodium, 9 μg/g; copper, 0.8 μg/g; iron, 20.1 μg/g; manganese, 13.1 μg/g; zinc, 1.8 μg/g (analysis by Norwest Laboratories, Winnipeg, Canada). AMF were virtually never observed in plants grown in potting soil alone.

Soil-inoculum was added to pots in a similar manner as in Chapters IV and V. Pots were then randomly assigned to each pot-composition category. After seeding with the appropriate species, pots were placed in a greenhouse with 130 $\mu mol.m^{-2}.sec^{-1}$ supplemental lighting. The method of introducing the background microflora of untreated soil-inoculum to pots with autoclaved inoculum is described in Chapter IV (except that the background microflora was pipetted directly into the soil around seedlings, as in Chapter V).

Pots were fertilized biweekly with 1/4 Hoagland's solution without phosphate, and the position of pots within the greenhouse was re-randomized 3 times during the course of the experiment.

Plants were harvested 61 days after seeding, and shoot biomass of each species was determined separately. I was unable to accurately separate roots from individual species in the competition treatments, so analysis is restricted to shoot biomass. A 0.3 g subsample of the root system from each pot was collected, to check for AMF-colonization.

4. Data Analysis

a) Shoot biomass

Each species was analyzed separately, and there were separate analyses for plants that received inocula from sites 1 and 3. Shoot biomass per pot (SB) was analyzed by two-way ANOVA, with the two fixed factors of inoculation treatment and pot composition. Shoot biomass from 6 plant/pot monocultures was divided by 2 to obtain a measure of the effect of 3 individuals on 3 conspecific individuals. This analysis also tested if intraspecific competition occurred at the chosen levels of plant density; if competition was present, SB of the 3 plants/pot monoculture treatment should have been significantly larger than SB of the 6 plants/pot treatment (divided by 2). This analysis also allowed the comparison of the competitive effects of conspecifics and different species. The significance of differences between individual categories of pot-composition was tested by the overlap of 95% confidence intervals.

b) Differences among species in the intensity of competition

The yield-suppression index (*YS*) was calculated for each pot containing a species-mixture or 6 plants/pot of conspecifics. As in the previous analysis, TSB of pots in the 6 plants/pot monoculture category were divided by two before analysis. YS_{ij} is a measure of the relationship between *SB* of species *i* in pot composition category *j* and mean *SB* of species *i* in monoculture (3 plants/pot). It is a measure of the intensity of competition (Aarssen, 1985):

$$YS_{ij} = SB_{ij}/\overline{SB}_{i(3plants/pot)}$$

As *YS* aproaches 0, competition intensity increases, and as it approaches 1, competition intensity decreases.

YS of all 3 species was analyzed together by 2-way ANOVA. The 2 factors

were inoculation treatment and pot composition. There were 9 categories of pot composition, incorporating 3 monoculture categories, and 6 competition categories. Six competition categories were required because each species of each mixture was considered to be a "target", and was placed in a separate category. For example, competition between *A. trachycaulum* and *H. jubatum* led to a separate *YS* for *A. trachycaulum* and for *H. jubatum*. The significance of differences between individual categories of pot composition was tested by the overlap of 95% confidence intervals. Yield suppression was analyzed separately for plants receiving inocula from sites 1 and 3.

D. Results

1. Effects of competition

Pot composition had highly significant ($p<0.001$) effects on SB. For each species, TSB in the 3 plants/pot treatment was significantly greater than in the 6 plants/pot treatment (divided by 2), indicating the presence of intraspecific competition in pots with 6 plants/pot (Table 16, Figures 21 and 22). Shoot biomass was also significantly lower in each interspecific competition treatment than in the corresponding 3 plants/pot monoculture treatment.

Shoot biomass of *H. jubatum* was similarly affected by *P. nuttalliana* and *A. trachycaulum*. *P. nuttalliana* also responded similarly to its 2 interspecific competitors. *A. trachycaulum*, however, was more strongly affected by competition with *H. jubatum* than with *P. nuttalliana* (Figures 21 and 22).

Unlike *H. jubatum* or *A. trachycaulum*, *P. nuttalliana* was more strongly affected by interspecific competition than by intraspecific competition.

Table 16. 2-way ANOVA of total shoot biomass (TSB). (A),inoculum from site 1; (B), inoculum from site 2.

(A). Site 1.

Source of variation[a]	Plant species	Mean square	F-ratio	df
PC	*A. trachycaulum*	0.568	48.7***	3
IT	" "	0.100	8.6**	1
PC x IT	" "	0.010	0.8	3
Error "	"	0.012		37
PC	*H. jubatum*	0.506	22.6***	3
IT	" "	0.158	7.1*	1
PC x IT	" "	0.027	1.2	3
Error "	"	0.022		40
PC	*P. nuttalliana*	0.242	31.3***	3
IT	" "	0.008	1.1	1
PC x IT	" "	0.008	1.1	3
Error "	"	0.008		39

(B) Site 2

Source of variation	Plant species	Mean square	F-ratio	df
PC	*A. trachycaulum*	0.488	35.4***	3
IT	" "	0.107	7.8**	1
PC x IT	" "	0.033	2.4	3
Error	" "	0.014		36
PC	*H. jubatum*	0.477	10.1***	3
IT	" "	0.003	0.1	1
PC x IT	" "	0.014	0.3	3
Error	" "	0.047		34
PC	*P. nuttalliana*	0.307	36.1***	3
IT	" "	0.001	0.1	1
PC x IT	" "	0.010	1.2	3
Error	" "	0.009		40

[a]PC, pot composition; IT, inoculation treatment.
*$p< 0.05$; **$p< 0.01$; ***$p< 0.001$

Figure 21. Effects of inoculum from site 1 on total shoot biomass of pots containing monocultures or mixtures. (A) biomass of *A. trachycaulum*; (B), biomass of *H. jubatum*; (C), biomass of *P. nuttalliana*. *Shaded bars*, untreated inoculum; *unshaded bars*, autoclaved inoculum; *A*, *A. trachycaulum*; *H, H. jubatum*; *P.*, *P. nuttalliana.*

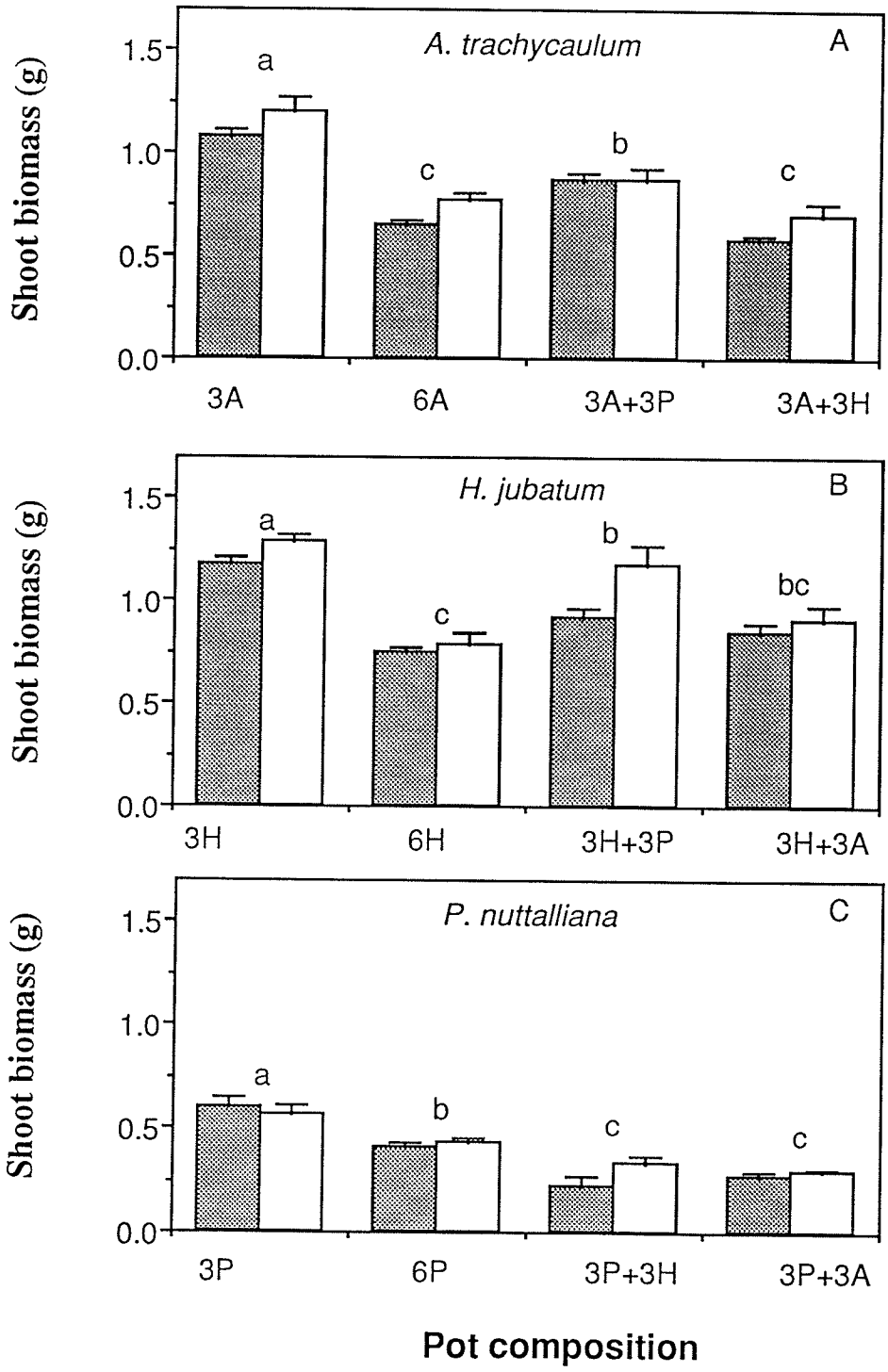
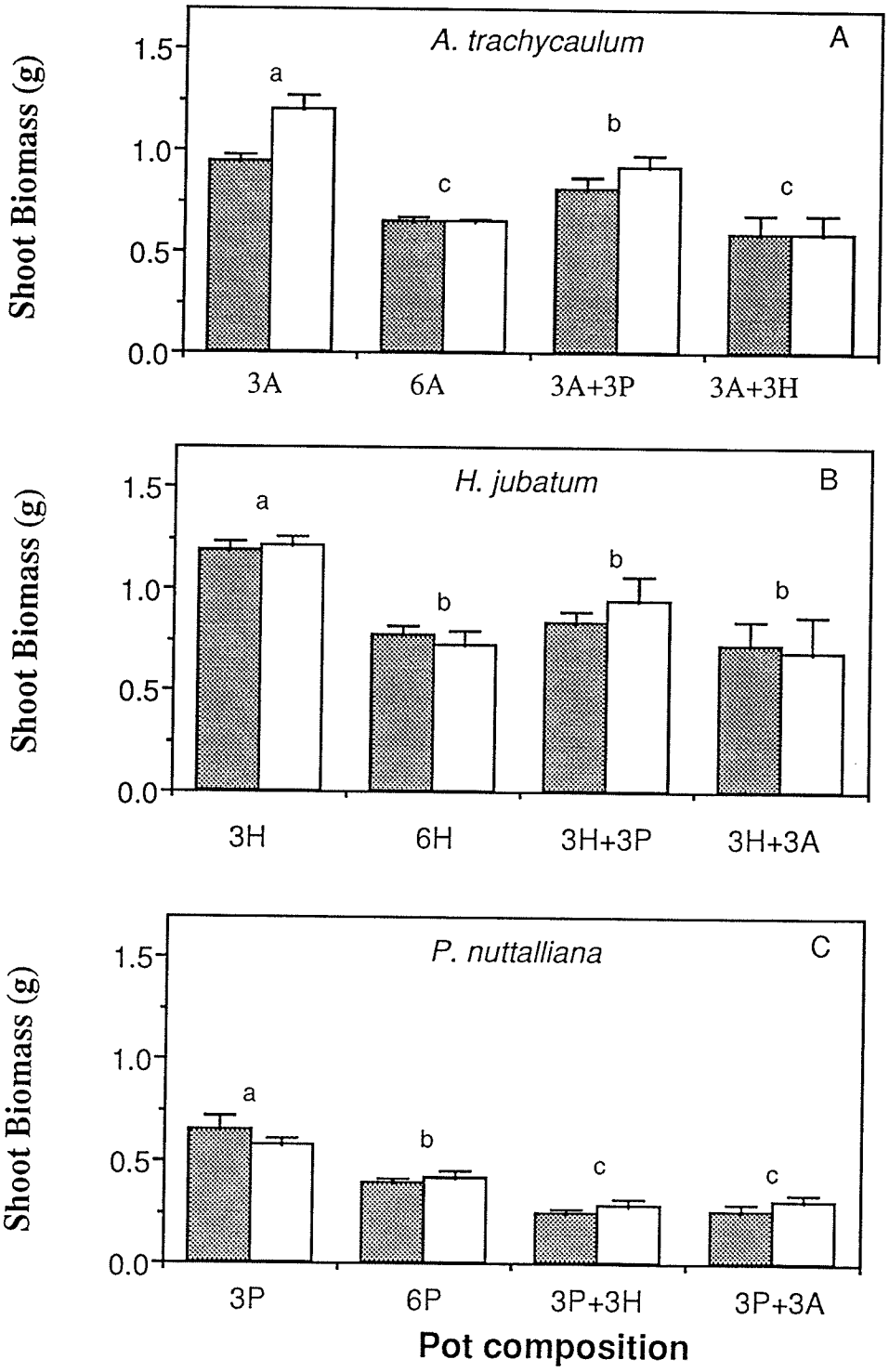

A. trachycaulum
A
Shoot biomass (g)
1.5
1.0
0.5
0.0
a
c
b
c
3A
6A
3A+3P
3A+3H
H. jubatum
B
Shoot biomass (g)
a
c
b
bc
3H
6H
3H+3P
3H+3A
P. nuttalliana
C
Shoot biomass (g)
a
b
c
c
3P
6P
3P+3H
3P+3A
Pot composition

Figure 22. Effects of inoculum from site 3 on total shoot biomass of pots containing monocultures or mixtures. (A) biomass of *A. trachycaulum*; (B), biomass of *H. jubatum*; (C), biomass of *P. nuttalliana*. *Shaded bars*, untreated inoculum; *unshaded bars*, autoclaved inoculum; *A*, *A. trachycaulum*; *H, H. jubatum*; *P.*, *P. nuttalliana.*


A. trachycaulum
A
Shoot Biomass (g)
1.5
1.0
0.5
0.0
a
c
b
c
3A
6A
3A+3P
3A+3H
H. jubatum
B
Shoot Biomass (g)
a
b
b
b
3H
6H
3H+3P
3H+3A
P. nuttalliana
C
Shoot Biomass (g)
a
b
c
c
3P
6P
3P+3H
3P+3A
Pot composition

2. Effects of AMF

Examination of ten root systems (three of *A. trachycaulum*, four of *H. jubatum*, and three of *P. nuttalliana*) revealed extensive AMF-colonization of plants exposed to untreated inocula from site 1 or 3. In the experiment described in Chapter V, infection by *Olpidium* spp., *Pythium* spp., or both, was common in roots inoculated with soil from site 1. Infection by these pathogens was not observed in the present study.

AMF from site 1 or 3 did not significantly affect shoot biomass of *P. nuttalliana* in monoculture or mixture (Table 16, Figures 21 and 22). However, growth of *A. trachycaulum* was significantly suppressed by AMF from site 1 or 3, and *H. jubatum* had less shoot biomass when colonized by AMF from site 1.

None of the interactions between inoculation treatment and pot composition were significant, indicating that the analysis of biomass did not detect any significant changes in the response of any of the species to competition when AMF were present. A significant interaction would have suggested that differences in biomass between pot composition categories are not consistent in pots with untreated versus autoclaved inoculum.

3. Variation in the intensity of competition

a) Site 1

The 2-way ANOVA of yield suppression (*YS*) in pots exposed to inoculum from site 1 revealed significant main effects of pot composition and inoculation treatment (Table 17), but the interaction term was not significant. The overall effect of untreated inoculum (as compared with autoclaved inoculum) was to decrease *YS*, indicating increased suppression of growth. However, the difference was quite small (autoclaved, 0.66 ± 0.02; untreated, 0.62 ± 0.02).

Table 17. Two-way ANOVA of *YS*. Plants were exposed to inoculum from site 1 (A) or site 2 (B).

(A) Site 1

Source of variation[a]	df	Mean Square	F-ratio
PC	8	0.184	13.5**
IT	1	0.056	4.08*
PC x IT	8	0.020	1.43
Error	83	0.014	

(B) Site 2

Source of variation	df	Mean Square	F-ratio
PC	8	0.171	7.60**
IT	1	0.007	0.31
PC x IT	8	0.035	1.55
Error	84	0.022	

[a]PC, pot composition; IT, inoculation treatment.
*$p<0.05$
**$p<0.001$

YS of *P. nuttalliana* in mixture, and *A. trachycaulum* (when competing with *H. jubatum*) was significantly more severe than *YS* of *H. jubatum* in mixture with either species (Table 18). Therefore, *H. jubatum* was the superior competitor when plants were exposed to autoclaved or untreated inoculum from site 1.

b) Site3

Inoculation treatment did not significantly affect *YS* when plants were exposed to inoculum from site 3 (Table 18). Although pot composition had highly significant effects on *YS*, the pattern among categories of pot composition was different than when plants were exposed to inoculum from site 1 (Table 18). For example, the suppression of *H. jubatum* by *A. trachycaulum* was equal to the suppression of *A. trachycaulum* by *H. jubatum*. Therefore, *H. jubatum* was not a better competitor than *A. trachycaulum*.

As in plants exposed to inoculum from site 1, *YS* was most severe in *P. nuttalliana* experiencing interspecific competition, and was least severe in *A. trachycaulum* or *H. jubatum* competing with *P. nuttalliana*.

E. Discussion

1. Effects of AMF on growth in monoculture

AMF significantly depressed shoot biomass of *H. jubatum* (when inoculum was from site 1) and *A. trachycaulum*. Similar effects were observed in previous experiments (Chapters IV and V). This response is in agreement with the observation in other systems (Hetrick *et al.* 1988a, 1990) that cool-season grasses with extensive root systems do not benefit, and may suffer from AMF-colonization.

P. nuttalliana was not significantly affected by AMF in this experiment. In the experiment described in Chapter IV, growth of *P. nuttalliana* was suppressed by AMF,

Table 18. *YS* of *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana*. (A), site 1; (B), site 2.

(A) Site 1

Target	Neighbour	YS[a]	n[b]
P. nuttalliana	*A. trachycaulum*	0.47 ± 0.06 *a*	11
P. nuttalliana	*H. jubatum*	0.49 ± 0.03 *ab*	12
A. trachycaulum	*H. jubatum*	0.55 ± 0.03 *ab*	12
H. jubatum	*H. jubatum*	0.62 ± 0.03 *bc*	12
A. trachycaulum	*A. trachycaulum*	0.62 ± 0.02 *bc*	11
H. jubatum	*A. trachycaulum*	0.71 ± 0.04 *cd*	12
P. nuttalliana	*P. nuttalliana*	0.71 ± 0.03 *cd*	12
A. trachycaulum	*P. nuttalliana*	0.76 ± 0.03 *d*	11
H. jubatum	*P. nuttalliana*	0.84 ± 0.03 *d*	12

(B) Site 2

Target	Neighbour	YS	n
P. nuttalliana	*A. trachycaulum*	0.44 ± 0.04 *a*	12
P. nuttalliana	*H. jubatum*	0.47 ± 0.04 *ab*	12
A. trachycaulum	*H. jubatum*	0.57 ± 0.05 *abc*	9
H. jubatum	*A. trachycaulum*	0.58 ± 0.09 *abc*	9
A. trachycaulum	*A. trachycaulum*	0.61 ± 0.03 *abc*	12
H. jubatum	*H. jubatum*	0.62 ± 0.03 *bc*	12
P. nuttalliana	*P. nuttalliana*	0.67 ± 0.03 *bc*	12
H. jubatum	*P. nuttalliana*	0.74 ± 0.05 *bc*	12
A. trachycaulum	*P. nuttalliana*	0.81 ± 0.04 *c*	12

[a]Mean (± SEM) index of the suppression of yield of the "target" species by the "neighbour" species. When the target and neighbour are the same species, *YS* refers to yield suppression in pots with 6 conspecific individuals. Means with the same letter are not significantly different ($p<0.05$). In order to facilitate interpretation, means are presented in order of decreasing competition intensity.

[b]number of replicates.

but the soil used had extremely low levels of phosphorus. The difference between plant gain (via gain of P from the mycorrhiza) and plant loss (via carbon loss to the fungus) may have been large in this low-nutrient soil. The soil used in the current study had moderate levels of P, and AMF may have been more able to transfer P to host plants. The ability of *P. nuttalliana* to limit AMF-colonization to lower levels than *A. trachycaulum* or *H. jubatum* may have restricted the loss of carbon to AMF, thus avoiding growth suppression.

2. Interspecific competition

Under my experimental conditions *P. nuttalliana* was the least competitive of the three species. This is in agreement with the results of Kenkel *et al.* (1991), who found that *P. nuttalliana* was a poor competitor when grown in mixture with *Poa pratensis* and *H. jubatum.* Our study, then, provides further evidence of the poor competitive ability of halophytes in non-saline soil, which may restrict their field distribution to saline soil (Silander and Antonovics, 1982, Bertness and Ellison, 1987, Kenkel *et al.* 1991).

It was initially predicted that *A. trachycaulum* would outcompete *H. jubatum.* In fact, after exposure to inoculum from site 3, the supression of *H. jubatum* by *A. trachycaulum* was equal to the suppression of *A. trachycaulum* by *H. jubatum.* Inoculum from site 1 led to competitive superiority of *H. jubatum* over *A. trachycaulum.* Therefore, the absence of *H. jubatum* in vegetation zones dominated by *A. trachycaulum* (Burchill and Kenkel, 1991) may be caused by other factors than competition. For example, litter accumulation underneath *A. trachycaulum* may inhibit germination of *H. jubatum* seeds (as in Molobsky and Augspurger, 1992). *H. jubatum* does not form rhizomes, and depends on seeds for dispersal. *A. trachycaulum*, in contrast, spreads vegetatively by rhizomes. It is also possible that the relative competitive abilities of these two species changes at other total plant densities (Law and

Watkinson, 1987), as well as under different experimental conditions (*e.g.* different soil volumes).

The observation that soil inocula from different sites have distinct effects on interspecific competition has implications for competition in the field. For species such as *H. jubatum* and *A. trachycaulum*, that appear to have similar levels of competitive ability, a difference in response to soil microorganisms may lead to a shift in the competitive balance, resulting in suppression of one of the species. It is important to note that the increased suppression of *A. trachycaulum* by *H. jubatum* occurred when plants were exposed to untreated *or* autoclaved inoculum. Therefore, the cause of this supression is non-mycorrhizal microorganisms. Evidently, there is a need for research on the effects of specific members of the soil microbial community on the intensity and outcome of plant competition.

AMF from site 1 also differed from site 3 in that untreated inocula from site 1 slightly, but significantly increased the intensity of competition. This disagreed with my hypothesis that AMF-colonization would result in a decrease in the intensity of competition. A similar effect was observed by Hetrick *et al.* (1994) in a study of competition between *Andropogon gerardii*, a strongly AMF-dependent grass, and two grasses that do not require AMF for growth in the field, *Koeleria pyramidata* and *Elymus canadensis*. AMF led to increased growth of *A. gerardii*, which led to increased intensity of competition with the other grasses. In our experiment this cannot explain the increased severity of competition when AMF were present, since all species reacted neutrally, or negatively to AMF. Perhaps the increased root density in the mixtures and 6 plant/pot monocultures led to increased colonization, and increased suppression of shoot growth. Alternatively, AMF may have increased the rate of uptake of P in mycorrhizal plants, resulting in increased intensity of competition for P in mixtures of

mycorrhizal plants. In Chapter IV, AMF-colonized plants had increased P concentration in shoot tissue.

The severity of suppression of *Puccinellia nuttalliana* by *H. jubatum* or *A. trachycaulum* was not alleviated when AMF were present, despite the suppression of the latter 2 species by AMF. Apparently the suppression was not large enough to affect the ability of the superior competitors to suppress *P. nuttalliana*. Therefore, AMF-mediated suppression of competitive dominants may not lead to competitive release of subordinate species if the disparity between competitive abilities is large, and the magnitude of the AMF-induced suppression of the dominant species is small. If correct, this may partially explain the lack of agreement between predicted and observed effects of AMF on the outcome of competition, which occurred in the current experiment, and has been previously observed (Miller and Allen, 1992).

This study has shown that non-mycorrhizal soil microorganisms can not only affect plant growth, but can also change the course of interspecific competition (*e.g.* difference in competitive suppression of *A. trachycaulum* by *H. jubatum*, depending on the source of untreated or autoclaved inoculum). Future studies should aim to further study this phenomenom; we need to know the identity of microorganisms that can produce such changes, and the potential of these changes to affect competitive relationships, and community structure in the field.

Chapter VII

AMF-colonization of plants in the Churchill region

A. Abstract

Churchill, Manitoba lies in a transition zone between the boreal forest and the low arctic. Although arbuscular mycorrhizal fungi (AMF) are common in the boreal forest, they are rare in the high arctic. The objective of this study was to determine if AMF are present in the Churchill region.

AMF were common in beach ridges, mud flats, and salt pans. They were present, but rare in lichen heaths. AMF, then, may be more common in the low arctic, especially in coastal areas, than was previously believed.

B. Introduction and methods

Arbuscular mycorrhizal fungi are considered to be less important in cold climates than in hot climates (Koske, 1987; Read, 1992). It is evident, however, that AMF are common in salt pans in the midst of the boreal forest of north-central Manitoba (Chapter II, Table 3), a region that has cold winters and short, cool summers (Figure 1). The principal objective of this chapter is to determine if the range of AMF extends to the region of Churchill, Manitoba. This is a more northern area, with a colder, more severe climate than the salt pans near Overflowing River.

Churchill lies on the western shore of Hudson Bay, a body of water that has pronounced effects on Churchill's climate. Inland regions at similar latitudes (60° N) in Manitoba and Saskatchewan have a climate typical of the boreal forest, with a resultant

vegetation dominated by black spruce (*Picea mariana*) or white spruce (*Picea glauca*). However, as one approaches the coast of Hudson Bay in the region of Churchill, the typical boreal vegetation becomes more diminutive in stature and finally is replaced by vegetation typical of the low arctic, dominated by low-growing woody shrubs such as *Dryas integrifolia*, *Betula glandulosa*, and *Rhododendron lapponicum*.

This transition may also be reflected in changes in mycorrhizal fungi. Most plants in the boreal forest are colonized by ecto- (EMF), arbuscular (AMF), or ericoid (ERMF) mycorrhizal fungi, depending on the taxonomic affinity of the host (Malloch and Malloch, 1981*a*, 1981*b*). Members of the Ericaceae are colonized by ERMF, conifers by EMF, woody shrubs by AMF or EMF, and herbaceous plants are either non-mycorrhizal or colonized by AMF.

In the arctic, however, AMF are rare (Kohn and Stasovski, 1990, Bledsoe *et al.* 1990), even in grasses and other plants that are usually colonized by AMF in temperate regions. Although several reasons for this absence have been postulated (*e.g.* short, cool growing season, protracted soil moisture, reduced rate of mineralization -Kohn and Stasovski, 1990), they have not been tested experimentally.

The low arctic, then, lies between the AMF-rich boreal forest and the AMF-depauperate high arctic. The objective of this study was to collect roots from the low-arctic Churchill region, to determine if AMF were present.

Roots were removed from plants in the following habitats, which are common in the Churchill region: lichen heath, beach ridges, mud flats, and salt pans near the Churchill river estuary. Roots were preserved in 50% ethanol, and stained with trypan blue (Chapter II). Fifty 5 mm root pieces from each sample were examined with a Leitz Orthoplan compound microscope, and the type of mycorrhiza (if any) in each root piece was recorded.

C. Results and Discussion

As expected, ERMF were common in lichen heath vegetation, and virtually all "hair-roots" of ericaceous plants were colonized by coils of ERMF (Table 19). EMF were common in woody non-ericaceous shrubs, but AMF were only found in *Dryas integrifolia.*

AMF, however, were common in plants in the other 3 habitats. In *Potentilla edegii* all of the root pieces were colonized by AMF. Arbuscules were frequently observed in roots from beaches, salt pans, and mud flats. Inspection of Table 19 reveals that woody non-ericaceous plants from these habitats were predominantly colonized by EMF, whereas forbs and grasses were colonized by AMF. This corresponds generally to colonization patterns in the boreal forest (Malloch and Malloch, 1981*a*,*b*), although AMF-colonization is more common in woody shrubs in the boreal forest.

It is clear, then, that the low arctic climate of the Churchill region does not prevent establishment of AMF. The lack of AMF in lichen heath vegetation may be due to the lack of suitable hosts, and not to unsuitable soil conditions, since AMF-colonized roots were present in *Dryas integrifolia.* This is apparently the first recorded case of AMF-colonization of *D. integrifolia*; other researchers have observed EMF, but not AMF-colonization (Miller and Laursen, 1978, Kohn and Stasovski, 1990, Bledsoe *et al.* 1990) in this species.

AMF, although commonly observed in this study, were largely confined to areas directly adjacent or very near to the shore of Hudson Bay. AMF in the low arctic, then, may have a limited distribution coinciding with the distribution of coastal hosts such as *Elymus arenarius*, *Potentilla edegii*, and *Puccinellia phryganodes*. Further study of a wider range of plant hosts in inland areas, however, is necessary to confirm this hypothesis.

Table 19. Mycorrhizal colonization in individual plants collected from the Churchill region.

Plant	% AMF[a]	%EMF[a]	%ERMF[a]	habitat[b]
Dryas integrifolia	28	64	0	LH
Dryas integrifolia	0	70	0	BR
Andromeda polifolia	0	0	82	LH
Rubus chamaemorus	0	60	0	LH
Rhododendron lapponi	0	0	58	LH
Ledum decumbens	0	0	70	LH
Betula glandulosa	0	38	0	LH
Elymus arenarius	88	0	0	BR
" "	42	0	0	BR
" "	44	0	0	BR
" "	0	0	0	BR
" "	0	0	0	BR
" "	0	0	0	BR
Senecio congestus	44	0	0	BR
Saxifraga oppositifolia	8	56	0	BR
Honckenya peploides	80	0	0	BR
Salix argentea	10	56	0	BR
Potentilla edegii	100	0	0	BR
Hedysarum mackenzii	10	0	0	B
Carex spp.	18	0	0	SP
Salicornia borealis	10	0	0	SP
Plantago maritima	88	0	0	SP
Puccinellia sp.	74	0	0	SP
" "	0	0	0	SP
" "	92	0	0	SP
" "	80	0	0	SP
" "	7	0	0	MF
Puccinellia phryganodes	36	0	0	MF

[a]percent of root pieces with colonization by AMF, EMF, or ERMF.
[b]LH, lichen heath; BR, beach ridge; SP, salt pan; MF, mud flat.

It is interesting to note that *P. phryganodes* from a mud flat was colonized by AMF. The presence of well-developed AMF in *P. phryganodes* from a wet mud flat suggests that soil moisture may not be important in the exclusion of AMF from the high arctic.

Since plants in the high arctic (Devon Island and Ellesmere Island) appear to be devoid of AMF, and plants in the Churchill region are commonly colonized by AMF, there is probably a point somewhere between Churchill and Devon Island that is the most northern extent of AMF colonization.

The presence of AMF in the boreal forest, and in the Churchill region indicates substantial ability among AMF species to tolerate long, severe winters, short growing seasons and cold soil temperatures. AMF, however, are evidently unable to survive the extremely severe climatic conditions of the high arctic.

In conclusion, AMF may be more common in the arctic than was previously believed, although it appears that EMF and ERMF are more important in lichen-heath communities. The importance of each of these fungi in ecological processes in the arctic remains to be determined.

Chapter VIII

General Discussion

A. Preamble

This thesis had three central objectives: (*i*) to determine the spatial and temporal distribution of AMF along a salinity gradient at an inland salt pan in north-central Manitoba; (*ii*) to compare the growth and salt tolerance of halophytic grasses with and without AMF; (*iii*) to assess the potential of AMF to influence competition among halophytic grasses.

B. Distribution of AMF

Evidently, AMF are widely distributed in inland salt pans. AMF frequently colonized roots of plants such as *Agropyron trachycaulum*, *Hordeum jubatum*, *Spartina gracilis*, and *Distichlis stricta*, that are very common in the salt pans. The large number of colonized roots observed in the Hordeum zone throughout the growing season indicates that the AMF species present possess considerable salt tolerance. However,the paucity of AMF-species in field soils and bait plants suggests that salt-tolerance may not be widespread among AMF.

This conclusion may not be valid. Little is known of the diversity of AMF in the boreal forest. Although many boreal species are colonized by AMF (Malloch and Malloch, 1981*a*, 1981*b*), few AMF-species may be capable of surviving the harsh winters and cool summers of the boreal forest. The low diversity of AMF observed in Overflowing River salt pans may reflect low diversity in the region, rather than low incidence of salt-tolerance among AMF-species. It is also possible that populations of *Glomus etunicatum* and *Glomus macrocarpum* at Overflowing River salt pans have greater salt tolerance than populations in non-saline soil. Previous studies have demonstrated substantial variation in populations of AMF (Stahl *et al.* 1990).

The rarity of AMF in the Puccinellia and Salicornia zones is evidence of an upper limit to the salt tolerance of AMF. AMF, then, have a moderate level of salt tolerance that appears to be very similar to the level of salt tolerance of plants in the Hordeum zone (*H. jubatum*, *D. stricta*, *Atriplex patula*, and *Grindelia squarrosa*). Interestingly, most of the plants that are capable of surviving in the Puccinellia and Salicornia zones (with the exception of *Puccinellia nuttalliana*) are from predominantly non-mycorrhizal families, and are rarely observed in non-saline soil. Perhaps the ability of such plants to survive without mycorrhizae permitted their evolution in soils too saline for AMF. The evolution of halophytes may have occurred predominantly in environments inimical to the survival of AMF. Plants of the Hordeum zone, however, probably evolved in environments where AMF were common.

This study has also shown that arbuscular mycorrhizal communities in the field have distinct structure (*i.e.* zonation) when a strong environmental gradient is present, a phenomenon not often documented in fungi. The close correspondence between fungal and plant zonation in this case may be caused by a common "threshold" of soil salinity. There may be particular constraints against the evolution of tolerance to soil salinity greater than this threshold; these constraints may be shared by widely disparate groups of organisms (in the case of Overflowing River salt pans, plants and fungi). A small number of vascular plants have evolved mechanisms to tolerate this high salinity, but mycorrhizal fungi may not have. Further studies are necessary to determine if the common boundary in zonation observed in this study is common in other saline communities, and if it extends to other organisms (*e.g.* invertebrates and bacteria).

C. Growth-response to AMF

The results of Chapter IV imply that *H. jubatum* does not benefit, and may suffer from AMF-colonization. It seems surprising, then, that plants such as *H. jubatum*

have not developed mechanisms of resistance to AMF-colonization. It is uncertain, though, if the growth suppression observed in greenhouse and growth chamber-grown plants occurs in the field. There are significant differences between the field and greenhouse environment; light intensity, soil volume, and variation in temperature, soil moisture, and other soil properties are all lower in greenhouse experiments. Furthermore, the method of inoculation used in my greenhouse studies required disruption of the networks of mycorrhizal hyphae found in field soil. The effects of these differences between field and greenhouse conditions on plant response to mycorrhizae may well be substantial (Read, 1992). Nevertheless, the negative effect of AMF on *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana*, is similar to the response of other cool-season grasses (Grime *et al*, 1987, Hetrick *et al.* 1990), and may reflect the response to AMF in the field. It is also possible that AMF-driven increases in P concentration in plant tissue contribute to plant survival and reproduction. Increased P concentration in seeds may improve the viability of seeds, and increase the frequency of successful seed establishment.

It is important to confirm the results of my greenhouse and growth-chamber studies in the field; this can perhaps best be accomplished by the use of fungicides such as benomyl. The results of Chapter V (*Pythium* infection, in particular), however, confirm that one should be very careful when interpreting the results of fungicide application to field soils. This is especially true in systems such as inland salt pans, where many of the plants (cool-season grasses) would be expected to react neutrally, or negatively to AMF. In systems where plants react positively to AMF, fungicide-induced suppression of pathogens can be distinguished from fungicide-induced suppression of mycorrhizae (Hetrick *et al.*, 1994).

In this thesis, I have concentrated on the effects of AMF on the cool-season grasses *Puccinellia nuttalliana*, *Hordeum jubatum*, and *Agropyron trachycaulum.* It

should be emphasized that these are not the only plants important in the Overflowing River salt pans; plants such as *Grindelia squarrosa*, *Sonchus arvensis*, *Spartina gracilis*, *Distichlis stricta*, *Plantago maritima*, and *Aster pauciflorus* are all common in the salt pans, and have poorly developed root systems. These plants may react positively to mycorrhizal colonization, and the presence of AMF may be an important factor in their establishment and growth in saline soil.

The importance of non-mycorrhizal microorganisms

It appears surprising that cool-season grasses have not evolved defenses against mycorrhizal colonization. Perhaps continued receptivity to AMF is related to increased host resistance to pathogens. However, this was not evident in the experiment described in Chapter V; root systems that were well-colonized by AMF sustained extensive damage from *Pythium*.

AMF-induced increases in protection against plant pathogens have been demonstrated in the past, but this may be dependent on the timing of AMF-colonization. If AMF colonize the root before pathogen attack, substantial protection may result. This was confirmed indirectly in the examination of root systems of *H. jubatum* (Chapter V). Although the same root could have infections of both AMF and *Pythium*, infections of both organisms were never detected in the same region of a root.

In the experiments described in Chapter V, soil inoculum was collected in June. This may be a time when there are few propagules of AMF, but many propagules of *Pythium*. Since Chapter II illustrated that AMF-colonization of *H. jubatum* begins in late April, it may well be that field-grown plants are well colonized by AMF before they are attacked by *Pythium*. AMF-induced protection from pathogens such as *Pythium* could then be a major reason for the lack of resistance to AMF in *H. jubatum* (and other cool-season grasses). There has been considerable research on *Pythium* and other soil-borne

pathogens in an agricultural context. The findings of Chapter VI indicate that soil-borne pathogens can have important effects on plants in natural systems as well. This appears to be a fruitful area for future research.

It was also evident from Chapter V that researchers should exercise caution when interpreting results of experiments that rely on soil inoculum. Non-mycorrhizal "control" plants do not always have a similar "background microbial flora" to mycorrhizal plants. This supports Koide and Li's (1989) conclusion that the best experimental design for mycorrhizal experiments compares plants exposed to AMF spores to plants exposed to "spore-washings".

The exigencies of mycorrhizal research, however, demand that soil inoculum be used when the volume of inoculum required is large (*e.g.* Allen and Allen, 1988), or when investigating AMF-species that produce low numbers of spores. Several suggestions seem appropriate: When soil inoculum is used, roots should be carefully examined at high magnification for the presence of soil pathogens, and AMF-colonization should be measured in as great detail as is practical. This should increase the ability to distinguish experimental effects caused by AMF activity (*i.e.* arbuscule formation),from effects caused by other fungi. Of course, this does not account for the potentially confounding effects of rhizosphere bacteria. The bulk of the research on rhizosphere bacteria has had the laudable goal of increasing the productivity of agricultural plants. The response of plants to rhizosphere bacteria in natural systems is worthy of attention, both in the context of direct effects on plant metabolism, and in the context of effects on mycorrhizal colonization and activity.

The results of Chapter V (*i.e.* non-mycorrhizal microorganisms can strongly affect the growth of *H. jubatum*) do not invalidate the results of Chapter IV (*i.e.* AMF lead to suppression of growth of halophytic grasses). Four experiments (Chapter IV -Experiments 1 and 2, Chapter V -soil E, and Chapter VI) documented growth

suppression of *H. jubatum* when colonized by AMF. This was observed in experiments using soil-inoculum collected in 1991, 1992, and 1993, using 4 different potting soil-mixtures. It was also observed in saline soil (Chapter IV), where the activity of many other microorganisms was likely supressed (as observed in Chapter V). I am confident, then, in concluding that AMF collected from the Overflow Bay region suppress the growth of *H. jubatum* under greenhouse or growth-chamber conditions. AMF-driven growth suppression of *A. trachycaulum* was also repeatedly observed. *P. nuttalliana* was only suppressed by AMF in one experiment (Chapter IV - Experiment 1). The reduced colonization of roots of *P. nuttalliana* may protect it from adverse effects of AMF-colonization. This leads to the interesting possibility that highly salt tolerant plants such as *P. nuttalliana* and *T. maritima* may have evolved mechanisms to limit AMF colonization because of the inability of AMF to benefit plant growth in highly saline soil. In highly saline soil the drain of carbon to ineffective mycorrhizae may result in decreased plant survival. It would be valuable to test this idea by examining the effects of AMF on a variety of "extreme" halophytes at a range of soil salinities.

AMF and interspecific competition

The lack of suppression of *P. nuttalliana* by AMF did not improve its competitive ability (Chapter VI). Despite being suppressed by AMF, *A. trachycaulum* and *H. jubatum* were highly effective in suppressing the growth of *P. nuttalliana.* Mycorrhizal colonization may not have dramatic effects on competition among species that do not differ greatly in their response to mycorrhizae. In the experiment described in Chapter VI non-AMF microorganisms had stronger effects on the outcome of competition than did AMF.

Arbuscular mycorrhizal fungi from site 1 increased the severity of suppression of *P. nuttalliana* by *H. jubatum*. In this case, AMF apparently decreased the competitive

ability of an inferior competitor, resulting in increased growth suppression. This response may be relatively common in grasslands that have a large number of cool-season grass species.

As discussed above, it should be emphasized that this thesis concentrated on the response of cool-season grasses to AMF. The outcome of competition involving plants such as *Grindelia squarrosa* and *Sonchus arvensis* may be very different when AMF are absent.

In conclusion, AMF are common inhabitants of roots of plants in moderately saline soil at inland boreal salt pans. They consistently suppress the growth of *A. trachycaulum* and *H. jubatum*. Although AMF did not affect interspecific competition between *A. trachycaulum*, *H. jubatum*, and *P. nuttalliana*, their prevalence in the field indicates they have the potential to affect ecological processes (*e.g.* succession) in inland salt pans. AMF isolated from salt pans may also be useful in the cultivation of AMF-responsive crops in saline soil.

AMF are able to sustain colonization in moderately saline soil. This is further evidence of the enormous range of environmental conditions that arbuscular mycorrhizal fungi can withstand, despite a low level of species-diversity. The next challenge is to determine if the effects of AMF on their hosts observed in this study are characteristic of other halophytes, and to discover if they reflect the relationship between host and fungus in the field.

The continuous gradient in soil salinity present in inland salt pans is a valuable experimental tool for studying the interactions between mycorrhizal fungi, their plant hosts, and soil salinity. The conspicuous zonation of mycorrhizal colonization observed lends particular credence to the conclusion that inland salt pans are fascinating and exciting environments for mycorrhizal research.

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Appendix A:

The following procedure was used to stain mycorrhizal structures in roots (Koske and Gemma, 1989):

1. Clear roots in 2.5% KOH at 70-90°C for 20-40 min.
2. Bleach in fresh H_2O_2 (3 mL 20% NH_4OH in 30 mL 3% H_2O_2) for 10-45 min.
3. Soak (after rinsing) in 1% HCl.
4. Stain in 0.05% trypan blue (in 500 mL glycerol, 450 mL H_2O, 50 mL 1% HCl).
5. Stain at 90°C for 60 min or more.
6. Destain and store in acidic glycerol.

Appendix B:

Spore Isolation

The following procedure was used to isolate spores from soil:

1. Suspend soil (usually 100 mL) in 2000 mL H_2O.

2. After thorough mixing, pour suspension (leaving behind coarse sand and gravel) through 0.5 mm and 0.212 mm sieves. Retain the filtrate.

3. Pour filtrate through a 53 μm sieve. Collect material that is retained by the sieve.

4. Resuspend retained material in 500 mL H_2O. Let particles settle out for 30 sec, and then pour material in suspension through the 53 μm sieve. Collect material that is retained by the sieve.

5. Pellet the soil in a centrifuge (GLC table-top centrifuge at 2000 rpm for 6 min.

6. Resuspend pellet in 48% sucrose. Centrifuge immediately for 30 sec (2000 rpm).

7. Pour off spores onto 53 μm sieve. Wash thoroughly and examine microscopically.

Appendix C:
Digestion of Plant Tissue

The following procedure was followed in order to digest plant tissue in preparation for elemental determination using atomic absorption spectrophotometry (Na, Cu) or colorimetry (P).

1. Dried tissue was ground and passed through a 1mm sieve.
2. Ground tissue was redried (50-60^{o}C) prior to digestion.
3. 0.2 g of tissue from each sample was weighed out.
4. 0.5 mL H_2S0_4 and 4 mL $HN0_3$ were added per 0.2g of sample.
5. Samples were mixed using a vortex.
6. $HN0_3$ was removed by boiling at 120^{o}C.
7. H_20_2 was added dropwise until solution cleared.
8. Sample was heated for 10 minutes.
9. Additional H_20_2 was added if required for clearing.
10. Sample was cooled and volume made up to 25 mL.

Note for Atomic Absorption Spectrophotometry: for Na determination, KCl must be added to suppress ionization. Final concentration is 2000 ug/mL.