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Chapter 7

MYCORRHIZAL FUNGI ASSOCIATIONS WITH FOUR SALT MARSH SPECIES

***Melissa Pratt-Zossoungbo*[*] *and Patrick D. Biber*[**]**
Current Address: National Oceanic and Atmospheric Administration National Ocean Service Policy, Planning and Analysis Division, MBO RM 13349 1305 East-West Hwy Silver Spring, MD, 20910
Department of Coastal Sciences The University of Southern Mississippi Gulf Coast Research Laboratory 703 East Beach Drive Ocean Springs, Mississippi U.S.A.

ABSTRACT

The value of salt marshes in reducing wave energy, enhancing sedimentation, stabilizing sediment, providing fisheries habitat, and serving as a food source for wildlife is well documented and widely recognized. Restoration efforts often consist of whole plant harvesting from natural habitats. This imposes strong disturbances to the harvested areas, as well as plant availability being limited to the growing season. Development and evaluation of ecologically sound and cost-effective restoration methods using nursery-grown marsh grasses will advance the current state of restoration science and improve restoration effectiveness in estuarine and coastal habitats.

The introduction of Vesicular-Arbuscular Mycorrhizal (VAM) fungi may have the potential to increase nursery production and the health of the plants produced. VAM colonization may increase plant growth, improve water transport, increase resistance to pathogens, and mediate transplant shock of saltmarsh plants. VAM are also associated with enhanced plant survival in stressful environments including: water-stress, salinity stress, and low nutrient availability.

Saltmarsh species *Juncus roemerianus, Spartina alterniflora, Schoenoplectus robustus,* and *Schoenoplectus americanus* plants of various ages were inoculated with commercial mycorrhizal inoculant. Colonization rates were monitored and plant growth and health were assessed by morphological measurements. The effect of inoculant on

[*] Corresponding author: Tel: 301 713 3070 x 138 Email: melissa.pratt-zossoungbo@noaa.gov
[**] Tel: 228 872 4200 Fax: 228 872 4204 Email: Patrick.biber@usm.edu

morphological changes was also compared with the effects that the addition of fertilizer, and less saturated soil conditions had on plant growth and health.

These studies showed that species *Juncus roemerianus* and *Schoenoplectus* spp. are capable of acquiring VAM colonization, while *Spartina alterniflora* did not become colonized in mature or seedling stages. The effects of VAM on plant morphology varied between species and by the age of the plants and inoculation generally only had a measurable effect in seedlings. Inoculation had significant effects on plant growth for *Schoenoplectus* spp., while the addition of fertilizer had more pronounced effects on growth for *Juncus roemerianus* and *Spartina alterniflora*.

Keywords: Mycorrhizae, Vesicular-Arbuscular Mycorrhizae (VAM), *Juncus roemerianus, Spartina alterniflora, Schoenoplectus* spp.

INTRODUCTION

1. Salt Marsh Loss and Restoration

The value of salt marshes in reducing wave energy, enhancing sedimentation, stabilizing sediment, providing fisheries habitat, and serving as a food source for wildlife is well documented and widely recognized (Adam 1990, Boesch et al. 1994). Typically, a salt marsh is classified as the marsh area closest to the coastline, and in general, varies from ≤1-15 miles in width. In the salt marshes of the Gulf of Mexico these marshes are regularly tidally flooded, flat, polyhaline areas dominated by salt-tolerant herbaceous plants and very few other species (Eleuterius 1972, Boesch et al. 1994). In Mississippi this community is dominated by *Juncus roemerianus* (black needlerush), and *Spartina alterniflora* (smooth cordgrass). Other species include *Spartina patens* (salt meadow cordgrass), *Distichlis spicata* (saltgrass), *Schoenoplectus americanus* and *Schoenoplectus robustus* (salt marsh bullrush and three square bullrush) (Eleuterius 1972).

The most dramatic coastal wetland loses in the United States are in the Northern Gulf of Mexico, especially Louisiana (Turner 1990). This area comprises 41% of all national wetlands and has had 80% of all wetland losses (Dahl 1990, Turner and Gosselink 1975). From 1955-1978, 12,700 ha of wetlands were lost in this area, the same land area as Rhode Island (Turner 1997). In 2000 an unprecedented loss of salt marsh vegetation in coastal Louisiana and other areas along the northern coast of the Gulf of Mexico was documented at a rate of 25-35 mi^2 (65-91 km^2) each year. This loss stands to threaten Louisiana's coastal ecosystem, infrastructure and economy (Stewart et al. 2001).

In Mississippi there were approximately 26,237 ha of mainland marsh identified in 1968, of which 24,853 ha were dominated by *Juncus roemerianus,* and *Spartina alterniflora.* Tidal marsh is most extensive in the Pascagoula and Pearl River areas, with areas of 5,400 ha and 3,522 ha respectively (Eleuterius 1972). These numbers have somewhat declined since then, especially due to Hurricane Katrina in August 2005. Although there is less significant degradation of saltmarshes in Mississippi than in Louisiana, it is still important to maintain healthy marshes that can act as the first line of defense in future storms.

Human activities in Louisiana saltmarshes represent about 12% of the total land losses (Britsch and Dunbar 1993), while the remaining 88% of loses can be attributed to "indirect impacts" resulting from reduction in sediment supply due to dredging, subsurface fluid

withdrawal, and from hydrologic alterations (Turner 1997). The loss of these valuable habitats gives urgency to protect, conserve and restore these important resources. In efforts to overcome the loss of these valuable habitats, saltmarsh restoration projects are on the increase. The development and evaluation of ecologically sound and cost-effective restoration methods using nursery-grown salt marsh plants will advance restoration science and improve restoration effectiveness in estuarine habitats. Most plants used in coastal restoration come from a limited number of commercial growers or they are obtained by removing them from already stressed coastal habitats. Existing nurseries are often limited by the number of plants they can produce due to the time it takes for those plants to reach adequate maturity for transplantation. Nursery plants also can face stressful conditions once planted at the restoration sites, such as salt stress and temperature stress. The introduction of symbiotic fungi in the nursery environment may have the potential to decrease turn-around time and to increase the health and tolerance of restoration plants.

2. Mycorrhizal Fungi

Mycorrhizae are symbiotic associations that form between the roots of most plant species and a fungus, and are known as vesicular-arbuscular mycorrhizae (VAM). The root fungus association is one of the most ancient forms of symbiosis, and plays a large role in the succession and maintenance of plant community diversity (Finlay 2005). This occurs in nearly 80% of terrestrial plant communities (Read et al. 1976, Malloch et al. 1980, Harley and Smith 1983, Hoefnagels et al. 1993, Wigand and Stevenson 1994), but has rarely been studied in submerged aquatic plants and saltmarsh plants in the U.S. (Wigand and Stevenson 1994).

VAM are endomycorrhizae in which the fungal hyphae penetrate the plant cell wall (Figure 1). VAM grow intercellularly and intracellularly by hyphae or mycelia in the root cortex and form structures called arbuscules and vesicles. Arbuscules are "tree-like" structures that originate from branches of the intraradical hyphae after the branch hypha penetrates through the cell wall. An arbuscule forms between the cell wall and plasma membrane (INVAM 2008). Vesicles are thin-walled lipid-containing bodies produced terminally from hyphae in the root cortex (Figure 1).

Hyphae originate from a single entry point and have limited growth. They form an "infection (or colonization) unit" that is regulated by host-fungus interactions. Hyphae have various morphologies and functions from "infective" hyphae to "absorptive" hyphae to "fertile" (spore-bearing) hyphae (INVAM 2008). Infective hyphae initiate new points of colonization on the same root, other roots of the same plant, or roots of adjacent plants (INVAM 2008). The external hyphae grow outside the root and into the surrounding soil and reproduce by producing spores inside and outside of the roots (Hoefnagels et al. 1993).

The external hyphae are the most dynamic component of the fungal symbiosis. They not only interact with their own host-plant roots, but a whole range of organic and inorganic substrates as well as with bacteria, fungi, soil micro- and meso-fauna and the roots of secondary host plants (Finlay 2005). They are 10 times finer than plant roots and the cost to the plant of constructing a unit length of hypha is at least 100 times less than that of constructing a similar length of root (Helgason and Fitter 2005).

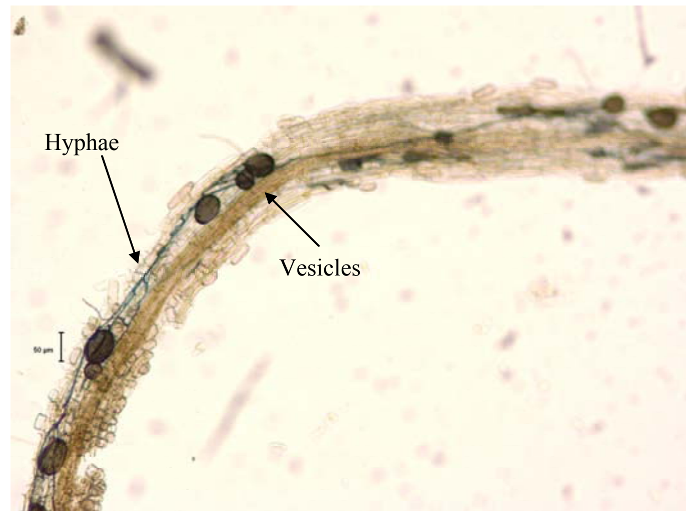


Figure 1. *Juncus roemerianus* root (100x) showing colonization with mycorrhizal fungi. Visible are hyphal structures and vesicles.

VAM spores are differentiated either in the soil or in the roots. Timing of onset of sporulation varies with species and also growing conditions (affecting the fungus directly and indirectly through host physiology). It often occurs within 3-4 weeks after onset of mycorrhizal colonization under almost any conditions. Spores begin to form after a threshold level of mycorrhizal biomass is achieved and increase dramatically after the roots cease to grow, but continue to function as a sink for carbon from the photosynthesizing tissue of the plant. The maximum spore production occurs near the middle to the end of the growing season (Mueller et al. 2004).

VAM are obligate symbionts because they acquire all of their carbon from the plant host (Helgason and Fitter 2005). The plant benefits from added surface area for nutrient uptake (Hoefnagels et al. 1993). Mycorrhizal colonization may improve water transport, resistance to pathogens, and transplant shock of plants (Dehne 1982, Linderman and Hendrix 1982). VAM are also associated with enhanced plant survival in stressful environments (Turner et al. 2000) including water-stress (Tobar et al. 1994), salinity stress (Allen and Cunningham 1983), and low nutrient availability (Wigand and Stevenson 1994).

One requirement for the survival of mycorrhizae in submerged systems is oxygen (Wigand and Stevenson 1994). Photosynthesis fuels the transport of oxygen to the roots via aerenchyma tissues and diffusion into the surrounding rhizosphere of submerged plant species allows VAM to survive (Sand-Jensen et al. 1982, Wigand and Stevenson 1994). Marsh soils are usually anaerobic. However, VAM have been found in saltmarsh plants in several countries including Pakistan (Khan 1974), India (Sengupta and Chaudhuri 1990), France (Boullard 1958), Great Britain (Mason 1928), and the Netherlands (Rozema et al. 1986).

3. Mycorrhizal and Plant Interactions

The majority of work on mycorrhizal fungi has focused on terrestrial plants and this symbiosis is well understood and documented in these plants (Aldon 1975, Malloch et al. 1980, Vogt et al. 1982, Porcel and Ruiz-Lozano 2004, Corkidi et al. 2005). However, the role of

VAM in saltmarshes is poorly understood. Of the studies looking at saline marsh environments, most have been to determine the presence or absence of the fungi, and most of these studies have looked at naturally occurring fungi and not VAM introduced through commercially available inoculants (Mason 1928, Rozema et al. 1986, Sengupta and Chaudhuri 1990, Hoefnagels et al. 1993, Brown and Bledsoe 1996, Aliasgharzadeh et al. 2001).

Many studies looking at vesicular-arbuscular mycorrhizae (VAM) in saline marsh environments have focused on the determination of the presence or absence of the fungi (Aliasgharzadeh et al. 2001, Carvalho et al. 2001 and 2004, Hildebrandt et al. 2001), while much of the work with VAM in terrestrial plants has examined the interaction of the plant and fungi with respect to increased growth and health of the plant. It has been shown that plants, both terrestrial and saltmarsh species, inoculated with VAM have increased growth and have greater leaf area (Al-Karaki 2000, Bhoopander et al. 2003). Greenhouse plants colonized with VAM have been shown to have more shoots per pot, increased biomass, higher survivability and increased chlorophyll content (Schubert and Hayman 1986, Al-Karaki 2000, Bhoopander et al. 2003, Asghari et al. 2005, Corkidi et al.2005).

VAM may also help alleviate the negative effects of conditions such as elevated salinity (Al-Karaki 2000, Feng et al. 2002, Ashgari et al. 2005), nutrient stress (Ietswaart et al. 1992, Johansen et al. 1992, Mader et al. 2000), and water stress (Miller 2000, Porcel and Ruiz-Lozano 2004). Carvalho et al. (2002) found that VAM were an important factor in the tolerance of saltmarsh plants to seawater tidal flooding, and additionally improved plant nitrogen acquisition. VAM have also been shown to decrease yield losses of plants in saline soils (Hirrel and Gerdemann, 1980). Thus, it has been shown that VAM may be able to reduce negative environmental conditions and produce larger, more robust plants.

Nutrients taken up by the VAM can lead to improved plant growth and reproduction. One study found that extramatrical mycelia (aggregates of hyphae) accounted for less than 20% of the total nutrient absorbing surface mass, but they contributed nearly 80% of the absorbing surface area of the plants (Rousseau et al. 1994). Another advantage attributed to VAM is access to phosphorus that the plant normally could not obtain. Phosphate (PO_4) availability in sediments is dependent upon free oxygen and redox potential (Mortimer 1942, Wigand and Stevenson 1994). VAM also aid in the uptake of nitrogen (Barea 1991). Experiments with ^{15}N showed that the external hyphae can derive ^{15}N from isotopically labeled ammonium salts (Ames et al. 1983, Johansen et al 1992, Tobar et al. 1994)

Mycorrhizal interactions with vascular plants are usually studied with respect to increased nutrient availability and plants with VAM are often more competitive and better able to survive. Sylvia (1992) showed the effect of inoculating grasses growing in native dunes with mycorrhizal fungi, comparing them to transplants placed on newly restored beaches. In the nursery, moderate amounts of VAM colonization were achieved. After transfer of these colonized plants to a low-nutrient beach environment, mycorrhizal colonization spread rapidly in plants and enhanced plant growth significantly compared to control plants without VAM. Compared to non-inoculated plants, VAM-colonized plants had 219% more shoot dry mass, 81% increased root length, 64% extra plant height, and 53% additional tillers after 20 months of growth on the beach (Sylvia 1992). Mycorrhizal colonization of plant roots has been shown to enhance plant health and healthy plants are important for successful salt marsh restoration projects. The interaction between VAM and saltmarsh species is an understudied area that has many implications for future restoration efforts.

In this chapter we examined the colonization success of four species of salt marsh graminoids (rushes, grasses and sedges) using commercially obtained VAM. We examined both mature and seedling stages of Black Needlerush (*Juncus roemerianus*), Smooth Cordgrass (*Spartina alterniflora),* Three Square Bullrush *(Schoenoplectus americanus),* and Saltmarsh Bullrush *(Schoenoplectus robustus)* for their ability to acquire VAM colonization. These are typical species that are used for restoration in Mississippi and elsewhere in the Gulf of Mexico.

The aim of these experiments was to determine the influence of inoculation with VAM fungi on plant growth and health of four species of saltmarsh plants at two different life stages in a greenhouse setting. We hypothesized that plants inoculated with VAM would have increased growth rates, better stress tolerance, and a better ability to acquire nutrients, which could lead to more effective and successful restoration efforts.

METHODS

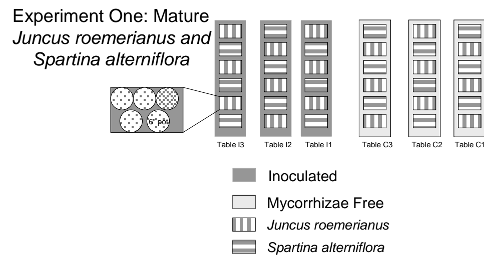
1. Experimental Design

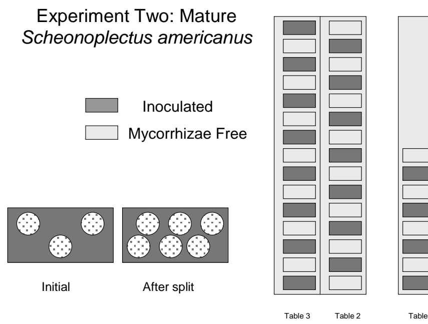
This study was divided into four experiments. Experiments one and two tested the responses of mature plants (>1 year age) and experiments three and four tested seedlings (< 6months age, germinated from seeds). Half of the plants in each experiment were inoculated with a general endomycorrhizal inoculant (Bioorganics, Oregon) containing a blend of eight types of endospores - *Glomus aggregatum, G. clarum, G. deserticola, G. intraradices, G. monosporus, G. mosseae, Gigaspora margarita,* and *Paraglomus brasilianum*, with a spore count of at least 50 spores per cubic centimeter, approximately 16,000 spores per kg.

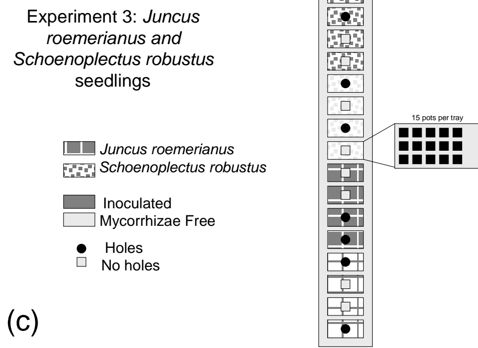
1A. Mature plants

In Experiment One, mature plants of *Spartina alterniflora* and *Juncus roemerianus* were purchased from a nursery (Aquatic Plants of Florida, Sarasota, FL) and planted into 6 inch pots (~1500cc) in a mix of 1:1 potting soil to cow manure and held outdoors under a 55% shade cloth for 4 months prior to inoculation in the late summer (Figure 2). Multiple 3 cm depressions were made in the soil near the roots of the plant and 5cc (~250 spores) of inoculant was distributed amongst the wells and covered with soil.

On day zero, three shoots per pot were marked and each shoot was measured for number of leaves, total height and green and brown height of all leaves on that shoot (n=45). Half way through the experiment leaf height was measured again at 120 days, while number of shoots and leaves per pot were counted at 180 days. All morphological metrics were determined at the end (300 days) of the trial (Figure 3). Five leaf samples for chlorophyll were collected at 120 and 150 days, and for tissue nutrients at 300 days. Plant biomass was determined at the start and end of the experiment. A dilute (10%) nutrient solution (Miracle Grow, 20N:20P:20K) was added to half of the plants in each treatment monthly from 120 - 240 days to compare the interaction between VAM inoculation with fertilization (Figure 3).







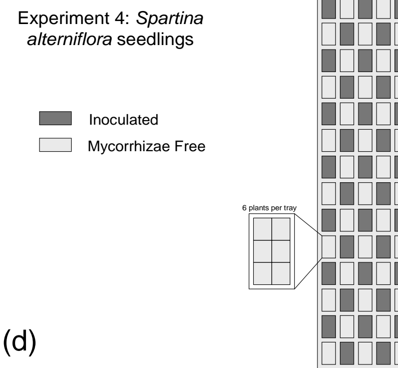


Figure 2. Experimental setup (a). Experiment One: Mature *Juncus roemerianus* and *Spartina alterniflora*, 45 plants per species in 18 trays each. Nine trays of each species inoculated with VAM, nine trays of each species left free of VAM (b.) Experiment Two: Mature *Schoenoplectus americanus,* 56 plants per treatment in 19 trays inoculated with VAM and 19 trays left free of VAM. (c.) Experiment Three: *Juncus roemerianus* and *Schoenoplectus robustus* seedlings, 8 trays per species, 15 pots per tray, 4 trays of each species inoculated with VAM and 4 trays left free of VAM (d.) Experiment Four: *Spartina alterniflora* seedlings, 210 plants per treatment in 35 trays inoculated with VAM and 210 plants in 35 trays left free of VAM.

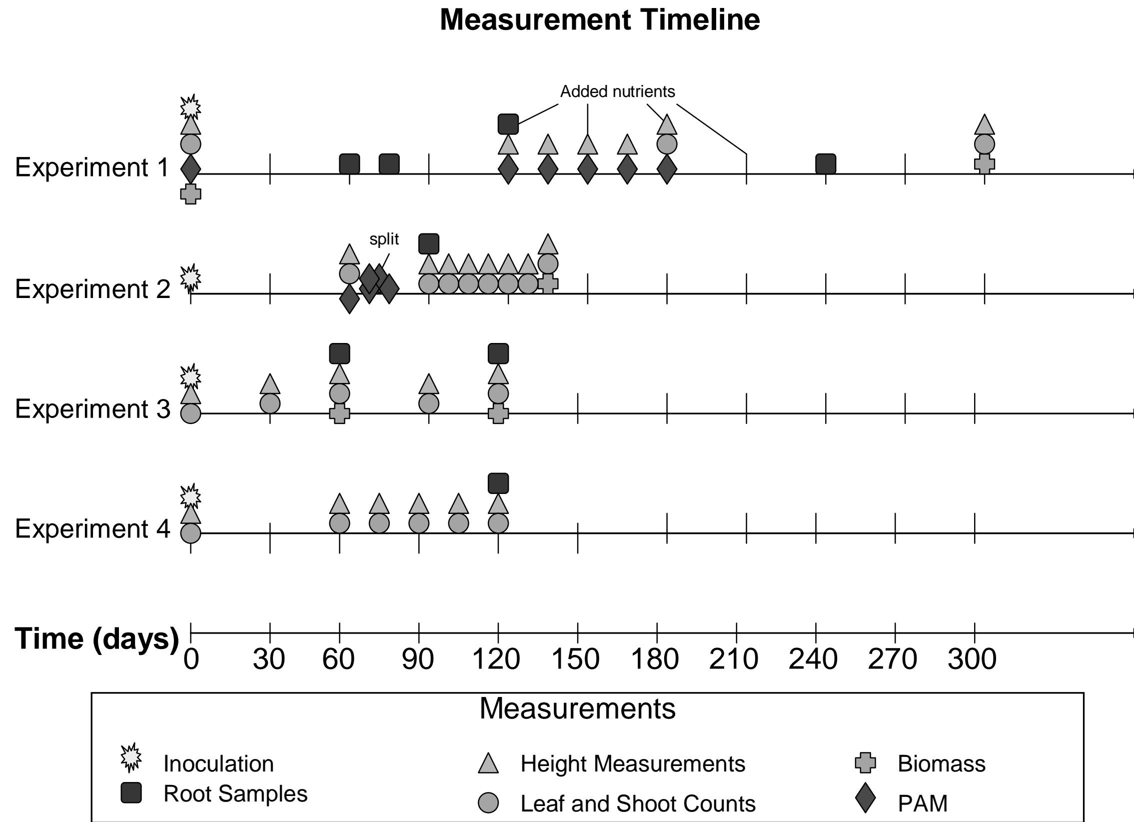


Figure 3. Timeline of measurements taken for Experiment One (Mature *Juncus roemerianus* and *Spartina alterniflora*), Experiment Two (Mature *Schoenoplectus americanus*), Experiment Three (Seedling *Juncus roemerianus* and *Schoenoplectus robustus*), and Experiment Four (Seedling *Spartina alterniflora*). Time 0 represents the point of inoculation with mycorrhizal inoculant.

In Experiment Two, mature *Schoenoplectus americanus* plants were propagated from native rhizome buds collected at the Gulf Coast Research Laboratory (GCRL), Ocean Springs, Mississippi, USA and rinsed free of sediments, then planted in 1:1 sand to topsoil mix, and allowed to grow for 6 months prior to inoculation in 6 inch pots in the early spring (Figure 2). Half of the plants were inoculated using a suspension of 188cc of inoculant (1/2 cup) per liter of tap water. This volume was evenly distributed (~5cc/plant, ~250 spores) and mixed into the top layer of soil.

Height of the tallest green leaf and the number of shoots and leaves per pot were recorded 60 days after inoculation (n=56). Four days later all leaves were trimmed, each plant was removed from the pot and cut in half to vegetatively propagate it. After the two halves were replanted, morphometrics were measured weekly for 60 more days to assess recovery. Biomass, chlorophyll and nutrients were assessed on twelve plants per treatment at 135 days (Figure 3).

1B. Seedlings

Seeds of *Juncus roemerianus*, *Schoenoplectus robustus* and *Spartina alterniflora* were collected at GCRL from wild populations. After germination, mycorrhizae-free seedlings were grown in peat pellets under controlled conditions until they were about 2-3cm tall (6-8 weeks) when they were transferred to the greenhouse.

In Experiment Three, seedlings of *Juncus roemerianus*, and *Schoenoplectus robustus* were transferred to 4 inch pots (~600cc) in 1:1 sand to topsoil mix when they reached a height of 5-10cm. Half the peat pellets were split and dipped into 5cc (~250 spores) of inoculant before planting in pots. 15 pots each were placed into 8 trays for each species (Figure 2). Holes were punched in the bottom of half of the trays (two trays per treatment) for drainage creating four treatments: control (C), control with holes (CH), inoculated (I), and inoculated with holes (IH). Drained trays promoted drier soil than the control trays, to test whether soil saturation affects the success of VAM colonization. Seedlings of both species were monitored monthly from 0 to 120 days for height, and number of shoots and leaves per pot (n=60). Biomass, and tissue samples were collected from 24 plants at 60 and 120 days (Figure 3).

In Experiment Four, *Spartina alterniflora* seedlings were planted into 2 inch wells (~125cc) in sterilized sand one month after germination. Sand had been sterilized in a Pro-Grow Electric soil sterilizer (model number SS15) for 2 hours at 94°C. Groups of six plants were placed into 35 trays per treatment (Figure 2). Seedlings were inoculated with 0.25cc (~12 spores) liquid suspension injected into the sand to the depth of the rhizosphere using a disposable pipette. The smaller volume used in the 2 inch wells standardized the amount of inoculant per unit volume of soil, to keep this treatment comparable with that received by the other three species in larger pots. Morphometrics were measured initially, half way (60 days), and then every two weeks until 120 days (Figure 3). No samples for tissues or biomass were collected in this experiment.

All trays in all four experiments were kept half full of freshwater by watering at least once per week.

2. Mycorrhizal Detection

Root samples were taken from haphazardly selected plants 60 - 240 days after inoculation (Figure 3), depending on the experiment. Experiment One plants were sampled at 60, 75, 120, and 240 days, with 5 - 10 samples per species were collected. Experiment Two root samples were collected from all 56 inoculated plants after 90 days and processed.

Experiment Three, 3 plants per tray were selected from both species and the root mass was processed and examined at 60 and 120 days. Experiment Four, the root mass of 8 seedlings was examined at 120 days only.

Roots of mature plants came from multiple locations on the root ball, inside and out, while the entire root mass of a seedling was harvested. Roots filled approximately half a 20ml scintillation vial; small, fine roots were preferred over larger, thicker roots. Roots were rinsed with tap water to remove sediment, cut into 2 - 4cm sections and stored in tap water in 20 ml vials until all samples were collected and were ready for analysis. If samples were not processed within 6 hours, samples were preserved in 50% ethanol. A modified procedure by Vierheilig et al. (1998) was used to clear and stain the roots. The chitin in the fungal cells stains blue-black while the plant material remains a reddish-brown making colonization easy to distinguish.

Stained roots were examined under a dissecting scope at 20-50x magnification to determine the presence of fungal spores, hyphae, arbuscules, and vesicles. Each sample was poured into a 10 x 10cm gridded square dish and all roots were examined for colonization using the visual method outlined by Giovannetti and Mosse (1980). We scored each root sample as positive if fungi were present, irrespective of the quantity or type of colonization. When colonization was detected, a representative root was moved to a glass slide and further examined using at 100-200x on a microscope with Nomarski interference contrast optics. After processing, the sample was returned to the vial and stored in acidified tap water.

3. Plant Tissues: Collection and Processing

For destructive tissue analyses, representative leaves were selected from each species and treatments. A 2.54cm (1 inch) long piece was removed from the middle of the leaf and stored frozen (-20°C) for later chlorophyll analysis. The remainder of the leaf tissue was dried and ground as outlined below prior to nutrient analysis.

To obtain biomass estimates, replicate whole plants for each species were harvested. All dirt was carefully rinsed from the roots and rhizomes, the below-ground (B) root ball was severed from the above-ground material, which was further divided into above- ground living (AL) and above-ground dead (AD) portions. Plant material was dried in at 65-70°C and then weighed to 0.001 mg accuracy.

For tissue nutrients (C:N:P), dried samples were ground in either a Wiley Mill using a 40 mesh sieve (425µm) or, for seedlings, the entire above-ground portion was ground in a Crescent Wig-L-Big oscillating ball mill. Ground samples were placed in 20 ml scintillation vials and stored in a dessicator at <50% relative humidity until analysis.

Duplicate samples, between 1 - 4 grams dry weight, were run on a Perkin-Elmer 2400 CN analyzer using acetinalide as a standard. Phosphate (P) concentration was determined after acid digestion of the sample, and free orthophosphate was determined colorimetrically (Granger and Iizumi 2001). A minimum of 3 replicate samples per treatment were run for each species and collection date.

Table 1. Mycorrhizal detection over time across three genera and two life stages of saltmarsh plants. In Experiment One some plants were sampled more than once.

Species	Weeks after inoculation	4	8	10	12	16	32	Percent colonized	Total number plants analyzed
Mature *Schoenoplectus americanus*					56/56			100%	56/56
Mature *Juncus roemerianus*			2/10	0/10		0/5	0/5	7%	22/45
Mature *Spartina alterniflora*			0/10	0/10		0/5	0/5	0%	24/45
Seedling *Schoenoplectus robustus*			0/12			6/12		25%	24/60
Seedling *Juncus roemerianus*			9/12			0/12		38%	24/60
Seedling *Spartina alterniflora*						0/8		0%	8/210

Table 2. Mean (±S.E.) responses of mature and seedling phases to control and VAM inoculation treatments of the three saltmarsh genera in the four experiments. No biomass or physiological variables were sampled in *Spartina* seedlings because of the high mortality.

Zone in marsh	High		Mid		Low	
Treatment	Control	Innoc	Control	Innoc	Control	Innoc
Zone in marsh	High		Mid		Low	
MATURE PLANTS	*Schoenoplectus*	(Expt 2)	*Juncus*	(Expt 1)	*Spartina*	(Expt 1)
Mortality (%)	0	0	0	0	0	0
VAM Inoculated (%)		100		6.6		0
BIOMASS (g dry wt)						
total	9.9 (0.65)	9.8 (0.89)	76.1 (6.20)	69.1 (2.68)	15.5 (2.52)	13.6 (1.91)
above	3.0 (0.13)	2.9 (0.28)	26.4 (3.86)	36.1 (7.57)	6.5 (1.27)	4.8 (0.05)
AL	2.5 (0.10)	2.4 (0.23)	14.9 (2.38)	19.7 (3.47)	2.3 (0.48)	1.6 (0.64)
AD	0.50 (0.049)	0.53 (0.066)	11.5 (1.53)	16.3 (3.47)	4.2 (0.86)	3.2 (0.69)
below	7.0 (0.50)	6.9 (0.59)	49.7 (9.62)	33.1 (4.94)	9.0 (1.27)	8.8 (1.96)
MORPHOLOGY						
# shoots/pot	12.6 (0.41)	12.8 (0.39)	39.9 (3.53)	37.7 (3.39)	26.9 (2.39)	21.3 (1.87)
mean # lvs/shoot	1.72 (0.023)	1.74 (0.021)	1.84 (0.053)	1.82 (0.043)	3.47 (0.109)	3.53 (0.113)
shoot height (cm)	61.2 (0.75)	61.7 (0.69)	64.3 (1.83)	66.7 (1.59)	42.2 (1.56)	42.6 (2.60)
PHYSIOLOGY						
%N	0.71 (0.075)	0.66 (0.070)	0.86 (0.148)	0.98 (0.210)	1.28 (0.090)	1.23 (0.105)
%P	0.015 (0.0008)	0.013 (0.0009)	0.011 (0.0018)	0.011 (0.0016)	0.030 (0.0033)	0.031 (0.0031)
N:P	19.7 (1.97)	21.7 (2.29)	34.3 (5.57)	33.6 (6.50)	22.3 (3.21)	17.4 (1.64)
Chl a (ug/mg)	0.518 (0.071)	0.582 (0.064)	0.836 (0.058)	0.933 (0.096)	1.425 (0.286)	1.329 (0.079)
Chl a (ug/cm^2)	12.4 (2.01)	13.4 (0.45)	25.8 (2.77)	28.0 (2.89)	40.0 (2.34)	36.7 (2.03)
Chl a:b	1.75 (0.069)	1.62 (0.157)	2.13 (0.083)	2.17 (0.070)	2.32 (0.104)	1.99 (0.210)

Table 2. (Continued)

SEEDLINGS	*Schoenoplectus*	(Expt 3)	*Juncus*	(Expt 3)	*Spartina*	(Expt 4)
Mortality (%)	37.5	25	2.1	0	94.8	71.0
VAM Inoculated (%)		25		37.5		0
BIOMASS (g dry wt)						
above	0.054 (0.008)	0.106 (0.015)	0.496 (0.075)	0.406 (0.081)		
MORPHOLOGY						
# shoots/pot	1.83 (0.049)	2.05 (0.033)	4.63 (0.187)	4.73 (0.262)	1.02 (0.258)	1.01 (0.246)
mean # lvs/shoot	2.69 (0.045)	3.16 (0.045)	2.31 (0.061)	2.36 (0.048)	1.97 (0.095)	2.11 (0.081)
shoot height (cm)	9.95 (0.235)	10.23 (0.228)	24.0 (0.70)	21.9 (0.62)	4.7 (0.34)	5.6 (0.23)
PHYSIOLOGY						
%N	1.42 (0.114)	1.38 (0.90)	1.50 (0.152)	1.66 (0.183)		
%P	0.0106 (0.0008)	0.0120 (0.0009)	0.0246 (0.0025)	0.0244 (0.0014)		
N:P	51.38 (5.60)	44.39 (2.96)	35.14 (3.90)	32.03 (4.00)		
Chl a (ug/mg)	1.149 (0.137)	1.514 (0.175)	1.487 (0.098)	1.700 (0.105)		
Chl a (ug/cm^2)	11.35 (1.36)	12.78 (2.25)	19.84 (2.47)	22.15 (1.65)		
Chl a:b	1.65 (0.142)	1.44 (0.115)	1.71 (0.111)	1.67 (0.052)		

Chlorophyll samples were first measured using digital calipers (0.01mm accuracy) to determine length and width (or diameter for *Juncus*) to calculate leaf surface area, and were also weighed to determine biomass. Chlorophylls were extracted from leaf tissues using 90% acetone after the methods outlined in Granger and Iizumi (2001). Chl *a* and chl *b* concentrations were calculated using the dichromatic equations for 90% acetone by Jeffrey and Humphry (1975). Chl *a* concentration was standardized to sample dry weight and leaf surface area. A minimum of five replicate samples were run for each treatment and species per collection date.

4. Statistical Analyses

Data were tested for normality and other assumptions of ANOVA in JMP ver 5. (SAS Institute, Cary, N.C.). Data that did not meet those assumptions were transformed (square root) or analyzed by non-parametric Kruskal-Wallis test. Where appropriate, tests were performed on mean responses by tray (independent replicate) rather than pot or plant. All post-hoc tests of significant results (α=0.05) were done using Tukey's Honestly Significant Difference (HSD) test.

RESULTS

Our studies showed that *Juncus roemerianus* and *Schoenoplectus* spp. are capable of acquiring VAM colonization, although the rates of colonization were generally low and differed by species and age. *Spartina alterniflora* did not become colonized in either mature or seedling stages. Colonization success in these three genera appears to correlate with the zone occupied in Mississippi saltmarshes; *S. alterniflora* is a low marsh species, *J. roemerianus* dominates the mid-marsh, while *Schoenoplectus* spp are restricted to the high marsh (Table 1).

1. VAM Colonization

In Experiment One, inspected at 60 days after inoculation, we found 20% of samples were colonized in *Juncus roemerianus*, while in *Spartina alterniflora* no colonization was found. In all subsequent examinations, no VAM were detected in either species (Table 2). In Experiment Two, mature *Schoenoplectus americanus*, all plants had some of the root material colonized by 90 days. In Experiment Three after 60 days no colonization was found in *Schoenoplectus robustus*, but 9 of the 12 *J. roemerianus* seedlings sampled were colonized. In contrast, after 120 days half of the 12 *S. robustus* seedlings were colonized, but there was no colonization in *J. roemerianus*. Eight *S. alterniflora* seedlings in Experiment Four examined at 120 days had no colonization (Table 2).

In summary, mature *J. roemerianus* and *S. alterniflora* plants showed little or no colonization, whereas 100% of the sampled mature *S. americanus* plants showed colonization. For seedlings, 37.5% of *J. roemerianus* and 25.0% of the *S. robustus* showed colonization, while *S. alterniflora* seedlings had no observable colonized roots. Control plants sampled showed no indication of VAM colonization.

Table 3. Results of statistical tests (P value) comparing means reported in Table 2 and sample sizes tested (n). Not all biomass fractions were tested in the mature plants.

Treatment	Control vs. Innoc		Control vs. Innoc		Control vs. Innoc	
MATURE PLANTS	*Schoenoplectus*	(Expt 2)	*Juncus*	(Expt 1)	*Spartina*	(Expt 1)
Total Biomass	0.9265	n=12	0.3614	n=3	0.5683	n=3
MORPHOLOGY						
# shoots/pot	0.6477	n=112	0.6546	n=30	0.0732	n=30
mean # lvs/shoot	0.4489	n=112	0.7761	n=30	0.6879	n=30
Shoot height	0.5967	n=112	0.3313	n=7	0.9056	n=7
PHYSIOLOGY						
%N	0.5962	n=24	0.6569	n=12	0.7174	n=12
%P	0.1853	n=24	0.9694	n=12	0.745	n=12
N:P	0.5123	n=24	0.9308	n=12	0.1905	n=12
Chl a concentration	0.5169	n=6	0.4062	n=10	0.4473	n=10
Chl a content	0.5913	n=6	0.5824	n=10	0.2929	n=10
Chl a:b	0.4996	n=6	0.7492	n=10	0.173	n=10
SEEDLINGS	*Schoenoplectus*	(Expt 3)	*Juncus*	(Expt 3)	*Spartina*	(Expt 4)
Biomass above	0.006	n=12	0.4228	n=12		
MORPHOLOGY						
# shoots/pot	0.0002	n=60	0.7535	n=48	0.6727	n=35
mean # lvs/shoot	<0.0001	n=60	0.4795	n=48	0.249	n=35
Shoot height	0.394	n=60	0.0291	n=48	0.0212	n=35
PHYSIOLOGY						
%N	0.7481	n=12	0.4872	n=12		
%P	0.2566	n=12	0.9406	n=12		
N:P	0.2816	n=12	0.5834	n=12		
Chl a concentration	0.1148	n=12	0.1533	n=12		
Chl a content	0.5929	n=12	0.445	n=12		
Chl a:b	0.2435	n=12	0.7557	n=12		

2. Plant Health and Growth

All measured responses to inoculation were not significant (α=0.05) in mature plants of the three saltmarsh species. In Experiment One, trays as a blocking effect were found to be insignificant, therefore only the main treatment effects of inoculation and fertilization were tested. There was no significant effect of inoculation on the final biomass, shoot height, number of shoots per pot, mean number of leaves per shoot of mature plants (Tables 2&3). Biomass increased over the 300 day and 135 day experiments respectively. In Experiment One, total biomass of *J. roemerianus* increased 49 - 55%, and *S. alterniflora* 10 - 22%, and with the majority of the biomass increase found in the below-ground fraction. In Experiment Two, the rate of increase in the number of shoots per pot of mature *S. americanus* was found to be significantly different between treatments (p=0.013) over 60 days after the plants were split into two and repotted. There was some indication of nutrient limitation in the control plants of the three species based on tissue nutrients and chlorophyll pigment analyses. Mean N:P ratios ranged from 17.4 in *S. alterniflora* to 34.3 in *J. roemerianus*, while average chlorophyll a:b ratios were between 1.62 and 2.32 (Tables 2&3).

Fertilization in Experiment One had a significant treatment effect on selected response variables, but had no significant interaction with VAM inoculation. There was a significant effect of fertilization on the mean height of *J. roemerianus* with plants in the fertilized treatments being taller than those in the unfertilized treatments (Tables 4&5). In *S. alterniflora*, total plant biomass was significantly greater in the fertilized treatments than in the control treatments, strongly supporting the importance of fertilizer enrichment on plant growth (Tables 4&5). Concurrently, there was also a significant enrichment of tissue N in the fertilized plants, compared to the controls (Tables 4&5).

Mortality of *Schoenoplectus robustus* and *Spartina alterniflora* seedlings occurred after the mid-point (60 days) due to overnight loss of heat in the greenhouse during the winter. More seedlings survived in the inoculated treatment, all of the *J. roemerianus*, 75% of the *S. robustus* and only 29% of the *S. alterniflora* (Table 3). In Experiment 3, the *J. roemerianus* seedlings showed significant differences with respect to soil saturation, while *S. robustus* seedlings exhibited significant differences with respect to inoculation. In Experiment Four, there was a significant effect of inoculation on survival of *S. alterniflora* seedlings ($P < 0.0001$) over 120 days; inoculated seedlings had significantly higher survival (1.5 per tray) than control plants (0.25 per tray).

Inoculated *S. robustus* seedlings had significantly greater above-ground biomass, and more shoots and leaves than control seedlings, indicating greater growth in the inoculated treatment. In contrast, both *J. roemerianus* and *S. alterniflora* had significantly greater shoot height in the inoculated treatment than the control seedlings (Tables 2&3). Neither tissue nutrients, nor chlorophylls differed significantly between treatments in Experiment Three, no samples were collected in Experiment Four because of the low number of surviving plants.

The drained trays in Experiment Three tested whether VAM were more beneficial to seedlings in less saturated soil conditions typical of the high marsh. For *J. roemerianus* at 120 days, there was significantly more above-ground biomass, more shoots per pot, and greater shoot height in trays that were drained compared to the saturated controls (Tables 4&5). In the high marsh species, *S. robustus,* soil drainage resulted in seedlings that had significantly fewer leaves per shoot at 60 days than control plants in the saturated trays (Tables 4&5). Significant interaction effects between inoculation and soil saturation were observed for P

content in *J. roemerianus* and N:P ratio in *S. robustus* leaves. In both instances, the interaction effect was a result of significant differences in the control (saturated) seedlings only (Tables 4&5).

3. Summary of Findings

The effects of VAM varied among species and by the age of the plants. Inoculation generally only had a measurable effect in seedlings, and was not obvious in mature plants of the same species. The mature plants in Experiment One and Two showed no significant effect of inoculation on biomass, morphology, or above-ground tissue constituents compared to control plants. The seedlings in Experiment Three and Four exhibited significantly enhanced biomass, morphometric, and tissue constituent responses to VAM inoculation across the three species. *J. roemerianus* seedlings in Experiment Three exhibited no effect of inoculation on above-ground biomass, shoots or leaves, but did show a significant effect on height. *S. robustus* seedlings showed significant differences between control and inoculated seedlings for the morphological factors of shoots and leaves, and biomass at 60 days, prior to die-back. Experiment Four showed significant effects of inoculation on *S. alterniflora* seedling survival.

The secondary treatments of fertilization also had varying effects on the plants. The mature plants in Experiment One showed significant effects of fertilization for height for *J. roemerianus,* and total biomass and tissue N content for *S. alterniflora*. The *J. roemerianus* seedlings in Experiment Three showed significant differences due to the addition of holes for drainage to some of the trays. The drained trays with less saturated soils had a significant effect on above-ground biomass, shoots per pot, and shoot height. For *S. robustus* seedlings the only effect the drained trays had was on leaves per shoot at 60 days.

DISCUSSION

As far back as 1928 it has been known that Vesicular Arbuscular Mycorrhizae (VAM) form symbiotic relationships with the roots of some saltmarsh plants (Mason 1928) and that they have a wide host range, but may have limited colonization potential in certain families (Harley and Smith 1983). Allen and Cunningham (1983) concluded that the importance of VAM varies from plants that are obligately mycorrhizal to non-mycorrhizal, while facultatively mycorrhizal plants can differ greatly in their responses to VAM. There are some reasons why certain species may not be as compatible as others including: production of diffusible or volatile antifungal root exudates, intrinsic resistance of the epidermis or cortex to colonization, and unfavorable soil conditions (Harley and Smith 1983, Hoefnagels et al. 1993, Corkidi et al. 2005).

Our studies showed that the species *Juncus roemerianus* and *Schoenoplectus* spp. are capable of acquiring VAM colonization, although the rates of colonization were low and differed among species and age. Mature *Schoenoplectus americanus* seemed to readily become colonized, while in *Schoenoplectus robustus* seedlings colonization was observed in half of the plants sampled. *Spartina alterniflora* did not become colonized in mature or seedling stages.

Table 4. Mean (±S.E.) responses of mature and seedling phases of the three saltmarsh genera to VAM inoculation and a second treatment factor in Experiments 1 (elevated nutrients) and 3 (reduced soil saturation by adding holes to trays).

Experiment 1				
Juncus	Control	+VAM	+NUTRIENT	+VAM +NUT
Total biomass (g)	75.6 (9.15)	104. 2 (16.20)	69.2 (2.68)	76.1 (6.20)
# shoots/pot	39.9 (3.53)	37.7 (3.39)	40.1 (3.95)	43.2 (6.65)
mean # lvs/shoot	1.84 (0.053)	1.82 (0.043)	1.91 (0.077)	1.91 (0.086)
Shoot Height (cm)	64.27 (1.83)	66.73 (1.59)	71.19 (0.81)	68.47 (1.20)
%N	0.84 (0.233)	0.67 (0.188)	0.88 (0.204)	1.28 (0.35)
%P	0.008 (0.0022)	0.01 (0.0024)	0.014 (0.0027)	0.013 (0.002)
N:P	41.9 (9.52)	27 (4.47)	26.7 (4.79)	40.1 (12.21)
Spartina				
Total biomass (g)	15.5 (2.51)	13.6 (1.91)	52.2 (5.20)	55.1 (10.31)
# shoots/pot	26.9 (2.39)	21.3 (1.87)	18.7 (2.44)	19.7 (2.97)
mean # lvs/shoot	3.47 (0.109)	3.53 (0.113)	3.29 (0.178)	3.24 (0.152)
Shoot Height (cm)	42.2 (1.56)	42.6 (2.6)	50.5 (6.57)	50.2 (7.2)
%N	1.07 (0.116)	1.08 (0.163)	1.49 (0.068)	1.38 (0.114)
%P	0.03 (0.0063)	0.032 (0.0059)	0.03 (0.0028)	0.031 (0.0027)
N:P	20.7 (4.73)	14.3 (1.69)	23.9 (4.69)	20.5 (2.25)
Experiment 3				
Juncus	Control	+VAM	+Holes	+VAM +Holes
Biomass above (mg)	306.2 (90.26)	375.9 (161.71)	685 (45.08)	435.5 (47.69)
# shoots/pot	4.17 (0.282)	3.96 (0.281)	5.06 (0.216)	5.5 (0.387)
mean # lvs/shoot	2.37 (0.101)	2.3 (0.065)	2.25 (0.071)	2.42 (0.069)
Shoot Height (cm)	22.6 (1.25)	21.1 (0.85)	25.3 (0.56)	22.7 (0.89)
%N	1.59 (0.289)	1.65 (0.294)	1.4 (0.118)	1.68 (0.245)
%P	0.030 (0.004)	0.024 (0.0019)	0.020 (0.0011)	0.025 (0.0021)
N:P	31 (6.29)	31.5 (5.41)	39.3 (4.54)	32.5 (6.41)
Schoenoplectus				
Biomass above (mg)	61.4 (8.15)	103.8 (26.47)	47.0 (14.68)	108.8 (16.86)
# shoots/pot	1.9 (0.056)	2.05 (0.06)	1.75 (0.079)	2.05 (0.028)
mean # lvs/shoot	2.84 (0.044)	3.23 (0.056)	2.54 (0.07)	3.1 (0.069)
Shoot Height (cm)	10.21 (0.315)	10.35 (0.368)	9.69 (0.348)	10.11 (0.273)
%N	1.39 (0.076)	1.4 (0.178)	1.46 (0.225)	1.35 (0.061)
%P	0.012 (0.0008)	0.012 (0.0016)	0.010 (0.0015)	0.012 (0.0008)
N:P	42.1 (2.64)	47.7 (4.88)	60.6 (9.83)	41.1 (3.22)

There is conflicting data in the literature that suggests that *S. alterniflora* may or may not acquire VAM colonization. Hoefnagels et al. (1993) looked at *S. alterniflora, Spartina patens, Spartina cynosuroides, Distichlis spicata,* and *J. roemerianus* in a North Carolina marsh and found that all species were mycorrhizal with the exception of *S. alterniflora.* They also conducted a greenhouse experiment with *S. alterniflora* and *S. patens* and again found no colonization of *S. alterniflora.* In contrast, McHugh (2004) used commercial inoculant to

infect *S. alterniflora* and *S. cynosuroides* in North Carolina and Connecticut salt marshes. *Spartina alterniflora* became infected, but colonization rates were low.

1. Mycorrhizal Colonization

Studies involving VAM require an evaluation of the plant roots to determine colonization. This can be to confirm if colonization has occurred, or a quantitative measure to see if morphological differences in the plant can be attributed to the quantity of colonization (Hepper 1977, Giovannetti and Mosse 1980). Usually, a portion of the host root tissue is visually examined to determine colonization (Giovannetti and Mosse1980). When collecting root samples for visual analysis, it is important to collect a representative sample of root material from the plants. There are several staining methods including the method by Phillips and Hayman (1970) using hot 10% KOH and 0.05% Tryptan blue in lactophenol; the method by Gerdemann (1955) using chloral hydrate as a clearing agent instead of hot 10% KOH; and the method by Vierheilig et al. (1998) that uses hot 10% KOH and a 5% Black Sheaffer Script Ink in Vinegar (5% acetic acid) solution. Other stains include cotton blue and acid fuchsin (Giovannetti and Mosse 1980, Vierheilig et al. 1998). These are all suitable stains, although the ink and vinegar stain (Vierheilig et al. 1998) provides a cheap, non-toxic alternative to some of the more noxious stains.

Colonization can be estimated in a multitude of ways after staining. Some methods involve randomly selecting root fragments from the sample and estimating the percent of each individual piece that is colonized, whereas others include looking at the entire sample and estimating percent colonization within the entire root cortex. Giovannetti and Mosse (1980) found that larger samples of roots are preferred to smaller samples, as is having as many replicates as possible, as colonization can vary greatly among plants and pots.

Another factor that can affect the detection of infection are differences amongst species of VAM in the way that they accept staining. Intraradical hyphae vary considerably in morphology and architecture within the family of Glomales, and only stain faintly, whereas Gigasporineae hypahe stain darkly (Mueller et al. 2004), both of which were contained in the commercial mix we used. There could have been much higher rates of infection that went undetected in all the species. Methods based on microscope analysis do not always allow systematic or functional separation of different fungal mycelia, or reliable separation of dead and live fractions of fungal biomass (Sylvia 1992). In addition, they can be difficult and time consuming.

In the future, visual examination coupled with other methods that such as testing for the presence of ergosterol could increase the positive identification of colonization by VAM. Ergosterol is a phospholipid component of fungal cell membranes that serves the same function that cholesterol serves in animal cells. This indicator can distinguish the different membrane lipids in the different fungi present in a sample (Larsen et al. 1998, Olsson 1999). Ergosterol has been used to determine the presence of fungi living in the roots of *Spartina alterniflora* (Padgett and Celio 1990) in the past with successful results. Phospholipid fatty acid analysis is seeing increased use in soil community analysis, as it has the potential to be a sensitive biochemical indicator capable of simultaneous estimation of, and distinction between, VAM and other fungal biomass (Olsson et al. 1995, Jansa et al. 1999, Olsson 1999).

Table 5. Results of 2-way ANOVA comparing means in Table 4. P value for each factor and the interaction are reported, along with results of Tukey's HSD test on significant results (α = 0.05).

Experiment 1				
Juncus	+VAM	+NUTRIENT	+VAM x NUT	Tukey's
Total Biomass	0.3059	0.1183	0.1102	
# shoots/pot	0.5217	0.9219	0.5515	
mean # lvs/shoot	0.231	0.8821	0.8737	
Shoot height	0.9241	0.0052	0.0787	Fertilized > Control
%N	0.6509	0.2101	0.2816	
%P	0.9684	0.0917	0.6343	
N:P	0.9296	0.9004	0.1078	
Spartina				
Total Biomass	0.9411	0.0002	0.697	Fertilized > Control
# shoots/pot	0.3747	0.0554	0.2019	Fertilized > Control
mean # lvs/shoot	0.9655	0.0943	0.6795	
Shoot height	0.1348	0.9944	0.9489	
%N	0.6774	0.0073	0.6122	Fertilized > Control
%P	0.7565	0.8801	0.924	
N:P	0.1929	0.2044	0.6833	
Experiment 3				
Juncus	+VAM	+Holes	+VAM x Holes	Tukeys
Biomass above	0.371	0.0373	0.1199	Dry > Wet
# shoots/pot	0.7105	<.0001	0.2761	Dry > Wet
mean # lvs/shoot	0.4891	0.9754	0.1368	
Shoot height	0.0282	0.0206	0.5648	Dry > Wet; Control > Innoculated
%N	0.5052	0.7454	0.6744	
%P	0.9331	0.1008	0.0361	Control Only: Wet > Dry
N:P	0.5921	0.4278	0.5333	
Schoenoplectus				
above	0.0083	0.7926	0.5921	Innoc > Control
# shoots/pot	0.0002	0.2027	0.2027	Innoc > Control
mean # lvs/shoot	<.0001	0.0006	0.1672	Innoc > Control; Wet > Dry
Shoot height	0.395	0.2531	0.6592	
%N	0.7587	0.941	0.6889	
%P	0.2631	0.2899	0.579	
N:P	0.2476	0.3241	0.0447	Control Only: Dry > Wet

2. Mycorrhizal and Plant Responses

Most differences were observed in the quickly growing seedling stages of *S. robustus* and *J. roemerianus*. Slower growing *J. roemerianus* seedlings showed fewer differences in morphological characteristic between treatments than *S. robustus*, but both seedling stages showed a greater influence of VAM than their adult counterparts. One reason for the only minor differences found between treatments with and without VAM addition could have been due to the low rates of colonization observed. Schubert and Hayman (1986) showed that growth responses are usually bigger when colonization by VAM is higher.

2A. Seasonal growth

One factor that could explain the low rates of VAM colonization observed may be related to the time of year the experiments were conducted. Studies have shown that the degree of mycorrhizal colonization in salt marshes is known to vary with season, both in intensity and in the formation of specific fungal structures (Cooke and Lefor 1990, Brown and Bledsoe 1996). Hildebrandt et al. (2001) found that degree of colonization is not constant during a plant's life cycle and varied with growing season. Boher et al. (2004) found the month in which samples were collected significantly correlated with VAM colonization levels. Carvalho et al. (2001) demonstrated the highest levels of colonization corresponded to the period of highest plant growth during summer, also confirmed by Ietswaart et al. (1992). Burke et al. (2002) showed that the lowest colonization occurred during plant senescence.

We suggest that this link between colonization success and seasonal plant growth might have contributed to why mature *Juncus roemerianus* and *Spartina alterniflora* in Experiment One were poorly colonized over the winter months when growth is at a minimum (Figure 4). *S. alterniflora* exhibits clear seasonal patterns increasing in biomass and shoot density during the spring and decreasing during late fall and early winter (Schubauer and Hopkinson 1984). Root colonization by VAM might have been suppressed as well as leaf growth and shoot formation, which would have resulted in a lack of significant differences between inoculated treatments and fertilized treatments. It is likely that different results would be obtained if this experiment were repeated over a season of higher growth (spring and summer).

For Experiments Two – Four, all plants were kept in a greenhouse where the daily temperature fluctuations were less extreme than outside temperatures experienced by the plants in Experiment One, especially during cooler months. Despite this, there was a malfunction with the greenhouse heater during the month of January that exposed *Juncus roemerianus* and *Schoenoplectus robustus* seedlings to freezing temperatures over a period of two days, which contributed to a die-back in the *Schoenoplectus* seedlings. In contrast the mature *Schoenoplectus americanus* (Experiment Two) was conducted over the summer months when plant growth is at its highest rate and we measured 100% VAM colonization.

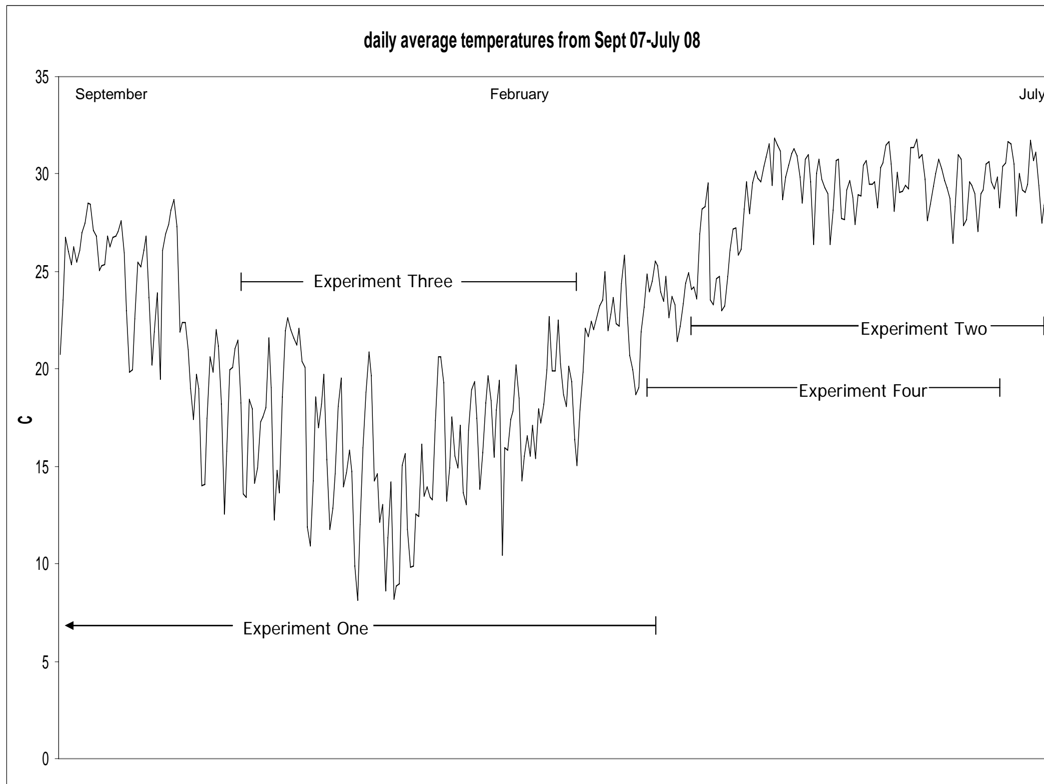


Figure 4. Average daily temperatures (°C) in the greenhouse for the duration of Experiment One, Experiment Two, Experiment Three, and Experiment Four.

2B. Soil nutrients

Soil nutrient concentrations can impact VAM colonization and therefore influence growth and health of the plants. McHugh (2004) looked at interactions between mycorrhizal colonization of roots and phosphorus (P) level in the soil on *Spartina* spp. Inoculated pots had greater root and shoot mass in low P treatments than did non-inoculated plants, while the opposite was true for high P conditions. In both *S. alterniflora* and *S. cynosuroides* the low concentration of P increased the root to shoot ratio and in both species the number of shoots per pot were significantly higher in the inoculated treatments. Comparing those results (McHugh 2004) with Experiment One, the plants that were given fertilizer and/or VAM showed little difference in height or number of shoots and number of leaves per shoot. However, fertilization did significantly increase biomass in *S. alterniflora.*

The soil in Experiments One - Three was probably not nutrient-limited initially which could have inhibited VAM colonization early in the experiment. It has been shown that VAM colonization can be suppressed in early stages by high levels of P in the soil (Johnson 1984). In contrast, plants that are nutrient stressed generally release more soluble carbohydrates in root exudates then fertilized plants, resulting in higher colonization of nutrient stressed plants (Johnson 1993). This suggests that plants growing in nutrient rich soils may have no need for VAM. Tissue N:P ratios can be used as an

indicator of plant nutritional status and help determine whether nutrients are limiting for growth. The average tissue concentrations are 2.5 – 4.5 % N and 0.2 – 0.75 % P, with corresponding N:P ratios of 6 – 12.5 (Munson 1998). The results obtained from all three species indicate that nutrients may have been limiting towards the end of the experiment, and that P-limitation was the main deficiency in both treatments despite fertilization.

Chlorophyll content has been shown to be influenced by colonization by VAM. Bhoopander (2003) showed that VAM had a strong influence on leaf chlorophyll content in *Acacia auriculiformis*. Inoculated plants had two-fold higher chlorophyll content than control plants. Feng (2002) in a greenhouse study with *Zea mays* seeds also found a significantly higher chlorophyll concentration in inoculated plants. *Schoenoplectus* spp were found to have below average chlorophyll concentration (<20 $\mu g\ cm^{-2}$), compared to the other two species. Chlorophyll was generally slightly higher in the inoculated plants in all experiments, as expected from previous studies. .

2C. Soil saturation

Soil saturation has the potential to affect the colonization success by these obligately aerobic VAM. In saltmarshes, soil saturation is affected by flooding and tidal inundation as well as soil composition and salinity. Bohrer et al (2004) and Carvalho et al (2004) found that VAM may not be limited by flooding and that they are tolerant of a wide range of soil moisture and flooding conditions. Juniper and Abbott (1993) found that spores of VAM germinated readily under water-logged conditions, but the growth of hyphae from pre-germinated spores was suppressed by the reduction in oxygen supply and the increased carbon dioxide levels in flooded soils. There are many instances of VAM in flooded soils, (Rozema et al. 1986, Cooke and Lefor 1990, Sengupta and Chaudhuri 1990, Secilia and Bagyaraj 1994, Wigand and Stevenson 1994, Brown and Bledsoe 1996, Miller 2000, Turner et al. 2000, Carvalho et al. 2001, Auge 2004, Boher et al. 2004, McHugh 2004) indicating that soil flooding may not be as harmful as the anoxic conditions that result from prolonged submergence. One interesting observation was noted by Brown and Bledsoe (1996), where they found VAM fungi in the aerenchymatous tissue of salt marsh plants, suggesting that VAM may tap into this tissue to survive in low oxygenated soils. In our experiments soil moisture was held high and nearly constant for all plants, except those in Experiment Three where there were holes cut into the bottoms of half of the trays to allow drainage. The seedlings in the trays with the holes cut in them had more colonization than the seedlings in the trays with no holes.

In Carvalho et al (2003) *Aster tripolium* seedlings were tested for the effects of salinity and flooding. Both increased salinity and increased water level significantly decreased the shoot dry weight of the seedlings. There was also a significant effect of salinity and water regime on both the percentage of total colonization of VAM and total colonized root length. But, the activity of VAM was only affected by salinity, supporting the conclusion that once established, salt marsh VAM might be adapted to flooding conditions. We used VAM isolated from terrestrial plant species, which might not have been well adapted to flooded conditions. It would be interesting to compare the two types of inoculant under flooded conditions and see if this is indeed true.

2D. Commercial vs. native mycorrhizae

There should also be further investigation to compare commercial inoculants with indigenous saltmarsh VAM to determine if in fact the saltmarsh VAM are better adapted to tolerate the unfavorable conditions of the intertidal zone. We examined salt marsh plants, but kept them in freshwater conditions. It would be interesting to compare VAM that are naturally found in saltmarshes with terrestrial VAM and commercial VAM to determine the effect of freshwater and saltwater on colonization. There have been numerous instances where VAM have been found in saline soils, but the data are somewhat contradictory. Aliasgharzadeh et al (2001) found no correlation between the number of spores and soil salinity or ion concentration, whereas Carvalho et al (2004) found that spores from salt marshes were more tolerant to salinity than spores from non-marsh environments. Alisagharzadeh et al (2001) also found that the percent of root length that was colonized by VAM decreased with increasing salinity in glycophytes. Allen and Cunningham (1983) found a difference between inland and coastal *Distichlis spicata* plants moved to hydroponic culture in their rate of colonization with the coastal plants having lower colonization rates under the same salinity. It would be interesting to see also if the converse was true; if VAM adapted to saline conditions was inhibited in freshwater.

With respect to using commercial mycorrhizal inoculant to increase nursery production of robust healthy saltmarsh plants, the data supports that VAM may help in the early stages of development, but is less useful in mature plants. Also, some species are more likely to acquire colonization, but that may not translate into increased growth or health for that species. This could be attributed to a multitude of factors including species compatibility, soil conditions, seasonality, fertilizer addition, and difficulties in determining colonization. Also, the type of VAM used in this experiment was not specific to salt marshes or flooded soils, which could have impacted its ability to colonize these salt marsh species. More study is needed to determine under what conditions VAM may provide a beneficial, cost effective approach to growing saltmarsh plants in a nursery setting.

CONCLUSION

There are many factors that can influence the infectivity of VAM among plant species, including several factors that were present in this study. The plants were kept in trays that simulated constant flooding, some plants were exposed to extreme temperatures in winter, and plants were planted in soil that probably was not nutrient limited, at least initially. It would have been useful to conduct these studies over the same season and to have grown all the plants in the same medium, as VAM can be affected by plant growth cycles as well as the season.

In Experiment One, fertilization proved to be more useful in respect to growth than inoculation with VAM. Inoculation seemed to help the seedling stages of faster growing species like *Spartina alterniflora* and *Schoenoplectus robustus.* In Experiment Three, when *Schoenoplectus robustus* seedlings were exposed to freezing temperatures, the surviving inoculated seedlings maintained greater numbers of shoots and leaves per shoot than the control seedlings. In Experiment Four, inoculated *Spartina alterniflora* seedlings had greater

survivability than seedlings that received no inoculant. Future investigation is needed to determine if the addition of VAM will indeed aid in saltmarsh restoration success.

There is a great deal of variation in the information known about VAM and saltmarsh plants with many conflicting studies and much variation and inconsistency amongst and between species with respect to colonization. Much more research needs to be done to conclusively determine under what conditions mycorrhizal fungi are best able to colonize saltmarsh plants. The different species and ecotypes of VAM elicit different effects on plant growth. There is also great variation amongst commercial inoculants in spore concentrations and the medium the spores are suspended in which can affect colonization (Corkidi et al. 2005). Further, we recommend that native saltmarsh VAM and commercially available VAM should be compared when possible to determine if there are differences between types of VAM in the colonization rates of saltmarsh plants. Saltmarsh restoration could benefit from a local supply of healthy, robust, native plants, and more research should be done to determine if inoculation with VAM would assist in this goal. Future studies are needed to elucidate the most appropriate species, soil conditions, and timing of VAM introduction that show a benefit to the plants in terms of increased growth, health, and ability to tolerate stressful conditions.

REFERENCES

Adam, P. (1990). *Saltmarsh Ecology*. Cambridge: University Press.

Aldon, E. F. (1975). *Endomycorrhizae enhance survival and growth of four-wing saltbush on coalmine spoils*. Washington D.C.: United States Department of Agricultural Forest Research.

Aliasgharzadeh, N., N. S. Rastin, et al. (2001). Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza, 11*, 119-122.

Al-Karaki, G. N. (2000). Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza, 10*, 51-54.

Allen, E. B. & Cunningham, G. L. (1983). Effects of vesicular- arbuscular mycorrhizae on *Distichlis spicata* under three salinity levels. *New Phytologist, 93*, 227-236.

Ames, R. N., C. P. P. Reid, et al. (1983). Hyphal uptake and transport of nitrogen from two 15 N labeled sources by *Glomus mosseae*, a vesicular- arbuscular mycorrhizal fungus. *New Phytologist, 95*, 381-396.

Asghari, H. R., P. Marschner, et al. (2005). Growth response of *Atriplex nummularia* to innoculation with arbuscular mycorrhizal fungi at different salinity levels. *Plant and Soil, 273*, 245-256.

Auge, R. M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science, 84*, 373-381.

Barea, J. M. (1991). Vesicular- arbuscular mycorrhizae as modifiers of soil fertility. *Advances in Soil Science, 15*, 1-40.

Bhoopander, G., R. Kapoor, et al. (2003). Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. *Biology and Fertility of Soils, 38*, 170-175.

Boesch, D. F., M. N. Josselyn, et al. (1994). Scientific assessment of coastal wetland loss, restoration and management in Louisiana. *Journal of Coastal Research, Special Issue No., 20*.

Boher, K. E., C. F. Friese, et al. (2004). Seasonal dynamics of arbuscular mycorrhizal fungi in differing wetland habitats. *Mycorrhiza 14*, 329-337.

Boullard, B. (1958). Les mycorrhizaes des especes de contact marin et de contact salin. *Revue de Mycologie, 23*, 282-317.

Britsch, L. D. & Dunbar, J. B. (1993). Land loss rates: Louisiana coastal plain. *Journal of Coastal Research, 9*, 324-338.

Brown, A. M. & C. Bledsoe (1996). Spatial and temporal dynamics of mycorrhizas in *Jaumea carnosa*, a tidal saltmarsh halophyte. *The Journal of Ecology, 84*, 703-715.

Burke, D. J., E. P. Hamerlynck, et al. (2002). Interactions among plant species and microorganisms in salt marsh sediments. *Applied and Environmental Microbiology, 68*, 1157-1164.

Carvalho, L. M., I. Cacador, et al. (2001). Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plant of the Tagus estuary (Portugal). *Mycorrhiza, 11*, 303-309.

Carvalho, L. M., P. M. Correia, et al. (2002). Effects of salinty and flooding on the infectivity of salt marsh arbuscular mycorrhizal fungi *in Aster tripolium* L. *Biology and Fertility of Soils, 38*, 137-143.

Carvalho, L. M., P. M. Correia, et al. (2004). Arbuscular mycorrhizal fungal propagules in a salt marsh. *Mycorrhiza, 14*, 165-170.

Cooke, J. C. & M. W. Lefor (1990). Comparison of vesicular- arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a coastal salt marsh in Clinton, Connecticut, USA. *Environmental Management, 14*, 131-137.

Corkidi, L., E. B. Allen, et al. (2005). Effectiveness of commercial mycorrhizal innoculants on the growth of *Liquidambar styraciflua* in plant nursery conditions. *Journal of Environmental Horticulture, 23*, 72-76.

Dahl, T. F. (1990). *Wetland losses in the United States 1780s-1980s*. Washington D.C.: Fish and Wildlife Service, United States Department of the Interior.

Dehne, H. W. (1982). Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology, 72*, 1115-1119.

Eleuterius, L. N. (1972). The marshes of Mississippi. *Castanea, 37*, 153-168.

Feng, G., F.S. Zhang, et al. (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza, 12*, 185-190.

Finlay, R. D. (2005). Mycorrhizal symbiosis: myths, misconceptions, new perspectives, and future research priorities. *Mycologist, 19*, 90-95.

Gerdemann, J. W. (1955). Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. *Mycologia, 47*, 619-632.

Giovannetti, M. & B. Mosse (1980). An evolution of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist, 84*, 489- 500.

Granger, S., & H. Iizumi. (2001). Water quality measurement methods for seagrass habitat. In F.T. Short, & R.G. Coles (Eds.), *Global Seagrass Research Methods*. (pp. 393-406) Amsterdam: Elsevier Science.

Harley, J. L & S. E. Smith (1983). *Mycorrhizae Symbiosis*. London: Academic Press.

Helgason, T. & A. Fitter (2005). The ecology and evolution of the arbuscular mycorrhizal fungi. *Mycologist, 19*, 96-101.

Hepper, C.M. (1977). A colorimetric method for estimating vesicular- arbuscular mycorrhizal infection in roots. *Soil Biology and Biochemistry, 9,* 15-18.

Hildebrandt, U., K. Janetta, et al. (2001). Arbuscular mycorrhizal colonization of halophytes in central European salt marshes. *Mycorrhiza, 10*, 175-183.

Hirrel, M. C. & Gerdemann, J. W. (1980). Improved growth of onion and bell pepper in saline soils by two vesicular- arbuscular mycorrhizal fungi. *Soil Science Society of America Journal, 44*, 654-655.

Hoefnagels, M. H., S. W. Broome, et al. (1993). Vesicular- arbuscular mycorrhizae in salt marshes in North Carolina. *Estuaries, 16*, 851-858.

Ietswaart, J. H., W. A. J. Griffioen, et al. (1992). Seasonality of VAM infection in three populations of *Agrostis capillaris* (Gramineae) on soil with or without heavy metal enrichment. *Plant and Soil, 139*, 67-73.

INVAM (2008). International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi [http://invam.caf.wvu.edu/index.html], West Virginia University.

Jansa, J., M. Gryndler, et al. (1999). Comparison of the lipid profiles of arbuscular mycorrhizal (AM) fungi and soil saprophytic fungi. *Symbiosis, 26*, 247-264.

Jeffrey, S.W. & P.W. Humphry (1975). New spectrophotometric equations for determining chlorophylls a, b, c in higher plants, algae and phytoplankton. *Biochem. Physiol. Pflanzen, 167*, 191-194.

Johansen, A., I. Jakobsen, et al. (1992). Hyphal transport of 15 N- labeled nitrogen by a vesicular- arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. *New Phytologist, 122*, 281-288.

Johnson, C.R. (1984). Phosphorus nutrition on mycorrhizal colonization, photosynthesis, growth, and nutrient composition of *Citrus aurantium. Plant and Soil, 80*, 35-42.

Johnson, N.C. (1993). Can fertilization of soil select less mutualistic mycorrhizae. *Ecological Applications, 3*, 749-757.

Juniper, S. & L. K. Abbott (1993). Vesicular-arbuscular mycorrhizas and soil salinity. *Mycorrhiza, 4*, 45-57.

Khan, A.G. (1974). The occurrence of mycorrhiza in halophytes, hydrophytes and xerophytes, of *Endogone* spores in adjacent soils. *Journal of General Microbiology, 81*, 7-14

Larsen, J., P. A. Olsson, et al. (1998). The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the saprotrophic fungus *Fusarium culmorum* in root-free soil. *Mycological Research, 102*, 1491-1496.

Linderman, R. G. & J.W. Hendrix. (1982). Evaluation of plant response to colonization of vesicular arbuscular mycorrhizae. A. Host variables. In N.C. Schenk (Ed.), *Methods and Principles of Mycorrhizal Research* (pp. 69-76). St. Paul: American Phytopathological Society.

Mader, P., H. Vierheilig, et al. (2000). Transport of 15N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytologist, 146*, 155-161.

Malloch, D. W., K. A. Pirozynski, et al. (1980). Ecological and evolutionary significance of mycorrhizal symbiosis in vascular plants. *Proceedings of the National Academy of Sciences, 77*, 2113-2118.

Mason, E. (1928). Note on the presence of mycorrhizae in the roots of salt marsh plants. *New Phytologist, 27*, 193-195.

McHugh, J. M. & J. Dighton. (2004). Influence of mycorrhizal inoculation, inundation period, salinity, and phosphorus availability on the growth of two salt marsh grasses, *Spartina alterniflora* Lois. and *Spartina cynosuroides* (L.) Roth., in nursery systems. *Restoration Ecology, 12*, 533-545.

Miller, S. P. (2000). Arbuscular mycorrhizal colonization of semi-aquatic grasses along a wide hydrologic gradient. *New Phytologist, 145*, 145-155.

Mortimer, C. H. (1942). The exchange of dissolved substances between mud and water in lakes. *The Journal of Ecology, 30*, 147-201.

Mueller, G. M., G. F. Bills, et al. (2004). *Biodiversity of Fungi, Inventory and Monitoring Methods*. Amsterdam: Elsevier Academic Press.

Munson, R. D. (1998). Principles of plant analysis. In Y.P. Kalra (Ed). *Reference Methods for Plant Analysis*. (pp. 1-24). Boca Raton: CRC Press.

Olsson, P. A. (1999). Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology 29*, 303-310.

Olsson, P. A., E. Baath, et al. (1995). The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research, 99*, 623-629.

Padgett, D. E., Celio, D. A. (1990). A newly discovered role for aerobic fungi in anaerobic salt marsh soils. *Mycologia, 82*, 791-794.

Phillips, J.M. & Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment. *Transactions of the British Mycological Society, 55*, 158–161.

Porcel, R. & J. M. Ruiz-Lozano (2004). Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *Journal of Experimental Botany, 55*, 1743-1750.

Read, D. J., H. K. Kovcheki, et al. (1976). Vesicular- arbuscular mycorrhiza in natural vegetation systems. *New Phytologist, 76*, 641-653.

Rousseau, J. V. D., D. M. Sylvia, et al. (1994). Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine. *New Phytologist, 128*, 639-644.

Rozema, J., W. Arp, et al. (1986). Occurrence and ecological significance of vesicular arbuscular mycorrhiza in the salt marsh environment. *Acta Botanica Nederlandica, 35*, 457-468.

Sand-Jensen, K., C. Prahl, et al. (1982). Oxygen release from roots of submerged aquatic macrophytes. *Oikos, 38*, 349-354.

Schubauer, J. P. & C. S. Hopkinson (1984). Above-and below- ground emergent macrophyte production and turnover in a coastal marsh ecosystem, Georgia. *Limnology and Oceanography, 29*, 1052-1065.

Schubert, A. & D. S. Hayman (1986). Plant growth response to vesicular- arbuscular mycorrhiza. XVI. Effectiveness of different endophytes at different levels of soil phosphate. *New Phytologist, 103*, 79-90.

Secilia, J. & D. J. Bagyaraj (1994). Selection of efficient vesicular- arbuscular mycorrhizal fungi for wetland rice: a preliminary screen. *Mycorrhiza, 4*, 265-268.

Sengupta, A. & S. Chaudhuri (1990). Vesicular arbuscular mycorrhiza (VAM) in pioneer salt marsh plants of the Ganges river delta in West Bengal (India). *Plant and Soil, 122*, 111-113.

Stewart, R. E., C. E. Proffitt, et al. (2001) *Abstracts from Coastal Marsh Dieback in the Northern Gulf of Mexico, Extent, Causes, Consequences, and Remedies*. Washington, D.C.: Pentagon Reports (A009893).

Sylvia, D. M. (1992). Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. In J. R. Norris, A. K. Varma, & D. J. Read (Eds). *Methods in Microbiology* (pp. 53-65). New York: Academic Press.

Tobar, R., R. Azcon, et al. (1994). Improved nitrogen uptake and transport from 15 N-labeled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. *New Phytologist, 126*, 119-122.

Turner, R. E. (1990). Landscape development and coastal wetland losses in the northern Gulf of Mexico. *American Zoologist, 30*, 171-197.

Turner, R. E. (1997). Wetland losses in the northern Gulf of Mexico: Multiple working hypotheses. *Estuaries, 20*, 1-13.

Turner, R. E. & Gosselink, J. G. (1975). A note on standing crop of *Spartina alterniflora* in Texas and Florida. *Contributions in Marine Science, 19*, 13-18.

Turner, S. D., & J. P. Amon, et al. (2000). Mycorrhizal fungi associated with plants in ground-water fed wetlands. *Wetlands, 20*, 200-204.

Vierheilig, H, A. P. Coughlan, et al. (1998). Ink and vinegar, a simple staining technique for arbuscular- mycorrhizal fungi. *Applied and Environmental Microbiology, 64*, 5004-5007.

Vogt, K. A., C. C. Grier, et al. (1982). Mycorrhizal role in the net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. *Ecology, 63*, 370-380.

Wigand, C. & J. C. Stevenson (1994). The presence and possible ecological significance of mycorrhizae of the submerged macrophyte, *Vallisneria americana*. *Estuaries, 17*, 206-215