Summer 8-2011

Mycorrhizal Colonization of Native Salt Marsh Plants on Mississippi's Gulf Coast and the Effects of Commercial Mycorrhizal Inoculants on Nursery-Grown Plants

Kathryn Rondot McBride
University of Southern Mississippi

The University of Southern Mississippi

MYCORRHIZAL COLONIZATION OF NATIVE SALT MARSH PLANTS ON MISSISSIPPI'S GULF COAST AND THE EFFECTS OF COMMERCIAL MYCORRHIZAL INOCULANTS ON NURSERY-GROWN PLANTS

by

Kathryn Rondot McBride

A Thesis
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

Approved:

Chester Rakocinski
Director

Jeff Lotz

Patrick Biber

Susan A. Siltanen
Dean of the Graduate School

August 2011

ABSTRACT

MYCORRHIZAL COLONIZATION OF NATIVE SALT MARSH PLANTS ON MISSISSIPPI'S GULF COAST AND THE EFFECTS OF COMMERCIAL MYCORRHIZAL INOCULANTS ON NURSERY GROWN PLANTS

by Kathryn Rondot McBride

August 2011

Salt marshes are important economically and ecologically to the Gulf Coast and other coasts worldwide. Due to human activities, many coastal salt marshes have been degraded or destroyed. Restoration efforts, through the replacement or addition of naturally occurring salt marsh plants, are taking place worldwide. Most restoration plants are raised in nurseries and are not ready for transfer to restoration sites for eight or nine months. Once the plants are at the restoration site many die due to transplant stress. Arbuscular Mycorrhizal Fungi (AMF) may be able to shorten the time the restoration plants need to stay in the nursery by increasing the plant's growth rate. AMF may also increase survival by decreasing transplant stress. To determine if *S. alternifora* and *J. roemerianus* are naturally colonized by AMF, wild plants were collected and examined for AMF colonization. Collections took place in the fall and spring to determine if there was seasonal variation in colonization. Spore-trap trays were utilized to determine if AMF colonization could be transferred from one naturally colonized wild collected plant to an un-colonized plant. A commercial AMF inoculant was tested to determine if the inoculant was able to successfully colonize salt marsh plants and to determine an effect on growth rates or biomass. The wild plant

collections showed that S*. alternifora* and *J. roemerianus* were naturally colonized by AMF and the colonization appeared to be seasonally influenced. The spore-trap trays did show that AMF colonization was able to transfer from one wild-collected colonized plant to an un-colonized plant. The commercial inoculant was not as successful at colonizing the salt marsh plants as the spore-trap trays were. The results suggest that naturally occurring AMF which are present in a salt marsh are more successful at colonizing plants and may be a better option for plant-based restoration projects in the future.

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF ILLUSTRATIONS

Figure

CHAPTER I

INTRODUCTION

Marsh Importance

Making up 41% of the total wetland area in the USA, Gulf of Mexico salt marshes play an important role within the coastal ecosystem (Turner & Gosselink, 1975). The marshes provide habitat for terrestrial animals including invertebrates, mammals, and migratory and non-migratory birds (Nybakken, 2001). Marshes also qualify as Essential Fish Habitat (EFH) for many commercially and ecologically important species along the Gulf Coast (Partyka & Peterson, 2008). As EFH, salt marshes contribute greatly to a fish species' productivity, and thus are protected under the Magnuson-Stevens Fishery Conservation and Management Act.

Emergent plants form the foundation of the saltmarsh ecosystem. The many species of plants help to stabilize marsh sediments and facilitate sediment accretion (Hopkins, Lugo, Alber, Covich, & Van Bloem, 2008). Marsh plants and their root systems help to build land by capturing sediment to and decreasing erosion. Saltmarsh plants have been shown to reduce wave energy by 90% and wave height by 70% (Bird, 2008).

Salt Marsh Sediments

Sedimentary dynamics within salt marshes provide an important ecosystem service. The sediments found in salt marshes are formed from sand to mud alluvial deposits (Rozema, Bijwaard, Prast, & Broekman, 1985). Salt marshes tend to occur on microtidal coasts, where wave action is just strong

enough to deposit sediment but not strong enough to dislodge the vegetation (Bird, 2008). They may be sheltered by embankments, or in the case of the Gulf Coast, protected by barrier islands.

The sediments of salt marshes act as a sink for nutrients as well as toxic materials such as metals, thus removing them from the surrounding environment (Keller, Lajtha, & Cristofor, 1998). Also due to the high level of organic matter input and the slow decomposition rate, salt marshes serve as a natural carbon sink. Due to high levels of nutrient input into marshes, they represent areas of active biogeochemical cycling mediated by microorganisms (Caravaca, del Mar Alguacil, Torres, & Roldan, 2005). The biogeochemical reactions within salt marsh sediments are unique due to anoxic and saline conditions (Pennings & Callaway, 1992). Although most of the sediment horizon is anoxic, the rhizosphere of marsh plants tends to be well oxygenated by the plant's roots (Rooney-Varga, Devereux, Evans, & Hines, 1997).

Several important groups of bacteria occur within salt marsh sediments, most of which are anaerobic. Three important groups of nitrogen processing bacteria occur in salt mash sediment; nitrogen-fixing, nitrifying, and denitrifying. Sulfur bacteria also reduce sulfur originating from decomposition of organic matter into a more usable form and give the marsh sediments a noticeable sulfuric smell (Rooney-Varga et al., 1997). In addition to bacteria, fungi are also important for microbial processing within marsh sediments.

Salt Marsh Plants

Salt marshes exhibit extremely high primary production rates due to the

vegetation which is present (Mitsch & Gosselink, 1993). In coastal salt marshes emergent plants tend to grow below the mean high tide level (Rozema et al., 1985). All salt marsh plants are classified as halophytes which are plants that can survive saline conditions (Zedler, 1984). The seaward zonation of plant flora in any salt marsh is related to spatial succession (Rozema et al., 1985) and is regulated by tidal inundation and soil aeration (Armstrong, Wright, Lythe, & Gaynard, 1985). The landward zonation is regulated mainly by competition for space and nutrients (Kiehl et al., 1997). A study by Pennings et al. (2005) showed that the salt marsh plant *Juncus romerianus*, successfully out-competes many other species in the marsh, but its range is limited by the physical stress of salinity and tidal inundation. In the same study, when *J. romerianus* was removed from a field site *Spartina alterniflora* was able to successfully spread into the vacant area, showing that it was competition by *J. romerianus* that drives *S. alterniflora* seaward. Although competition obviously affects salt marsh plants, two of the biggest stress factors, regardless of zonation, tend to be salinity and inundation (Rozema et al., 1985).

The salinity regime of coastal salt marshes can be detrimental to plants for several reasons; it can lead to Na and Cl toxicity, interfere with nutrient uptake, and lower external water potential. Salt marsh plants may possess several adaptations which make them able to tolerate high salinity or limited freshwater inputs (Pessarakli, 2002). To avoid Na and Cl toxicity, ions can be excluded at the root, secreted from salt glands, or taken up and concentrated in leaves before being shed (Rozema et al., 1985). By pumping oxygen from leaves down

to the roots, the rhizosphere remains oxic even in the harsh anoxic salt marsh sediment (Pessarakli, 2002). To maintain osmotic balance, plants can produce synthetic osmolytes or take up inorganic salts from the environment (Rozema et al., 1985). Salt marsh plants can also employ C4 photosynthesis, with uses the limited supply of freshwater more efficiently. Many of these plants also reproduce vegetativelly through clonal growth; and this may be an adaptation to reduce the amount of resources allocated to sexual reproduction (Pessarakli, 2002).

The salt marsh zonation of the northern Gulf Coast is unique compared to marshes on the Atlantic and Pacific coasts of the United States. The zonation of a "typical" southern marsh may begin with a *S. alterniflora* zone in the low marsh. This zone usually transitions into a dominant *J. romerianus* zone in the higher marsh. Behind this zone, there may be salt meadow zone and (depending on season and temperature) an accompanying salt pan, before transitioning to another sea meadow zone. The zone closer to upland can be a mixed community of *Schoenoplectus americanus, Schoenoplectus robustus, Spartina patens,* and *Distichlis spicata*, followed by terrestrial upland plants. In some southern marshes, the *J. romerianus* and *Schoenoplectus sp.* zones may be reversed in proximity to the tide (Odum & Barrett, 2005).

An Atlantic seaboard marsh may have some of the same plant species but in different distribution patterns. The low marsh of the Atlantic coast may consist of *S. alterniflora*, followed by *S. patens*, with the high marsh colonized by *D. spicata* (Odum & Barrett, 2005).

Pacific marshes have a unique vegetation pattern distinctive from that on

other coasts. The low marsh of the Pacific may consist of *Spartina sp.* followed by *Schoenoplectus sp.* Upland from those species some *Salicornia spp., Jaumea carnosa* and *D. spicata* may be present. The higher upland may be vegetated by *Cotula coronopifolia* and *Limonium californicum* (Odum & Barrett, 2005).

Salt Marsh Restoration

The California Department of Fish and Game defines a degraded wetland as being, “A wetland that has be altered by man through impairment of some physical property and in which the alteration has resulted in a reduction of biological complexity in terms of species diversity of wetlands-associated species which previously existed in the wetlands area.” (Zedler,1984, p. 2). Most salt marsh wetlands in the US have been subjected to decades of anthropogenic modifications including channeling, dredging, and hydrological alterations (Turner, 1997), and the Gulf Coast has been especially vulnerable. As a result of modifications, many of the marshes on the Gulf Coast have been degraded or destroyed. From the 1780s up to the 1980s loss of Gulf Coast marshes comprised 80% of the total wetlands losses in the US (Dahl, 1990). Sea-level rise in combination with subsidence has also contributed to the loss of large amounts of marsh on the Gulf Coast. Projected estimates of mean and maximum worldwide salt marsh loss due to sea-level rise are 20% and 45% by 2100 (Craft et al., 2009).

In response to the degradation of Gulf Coast marshes and others nationwide and increasing recognition of their intrinsic ecosystem service value, restoration efforts have been made over much of the coastal wetland areas

across the United States (Simenstad, Reed, & Ford, 2006). Restoration is defined as “returning a system to its predisturbed condition” (Zedler, 1984, p.3). The process of restoration may consist of biological remediation and/or biological augmentation. Remediation is the process of using organisms to remove toxins or excess nutrients from a polluted environment, thereby making it more suitable for naturally occurring species. Augmentation is the use of organisms to restore essential materials, nutrients, or natural habitat to an ecosystem (Campbell & Reece, 2008). Most salt marsh restoration projects focus on augmentation, by restoring or replacing naturally occurring plant species (Campbell & Biber, 2009). This is because plants are vital to the stability and evolution of coastal landscapes (Bird, 2008).

The majority of the plants used in restoration projects are grown in commercial nurseries. However, the number of plants that nurseries can produce is currently limited due to the time needed for plants to reach sufficient maturity for transplantation, which is usually estimated to be around six months. Arbuscular mycorrhizal fungi (AMF) may foster plant growth and shoot density in the nursery, thereby decreasing turnover time and also lowering transplant stress (Campbell & Biber, 2009).

Role of Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are members of the phylum Glomeromycota, one of seven phyla of fungi (Redecker, 2008). There are approximately 250 species of AMF which until recently were assumed to be redundant in their function (Bever, Schultz, Pringle, & Morton, 2001). They were

formally grouped in with Zygomycota but were moved to a separate phylum due to their lack of zygospores. All Glomeromycota are obligate symbionts with non-septate hyphae and relatively large multinucleate spores. Considered to be anamorphs, no evidence of sexual reproduction has been found (Redecker, 2008). In laboratory trials many species in this phylum have shown low plant host specificity but in nature AMF seem to form communities specific to plant type or location. Experiments have shown distinct AMF communities in legume versus non-legume plants and these plant-AMF combinations show specific relationships (Scheublin, Ridgway, Young, & van der Heijden, 2004). There have also been experiments showing the AMF communities found with pepper plants are unique to that group (Turkman, Sensoy, Demir, & Erdinc, 2008).

Arbuscular mycorrhizal fungi are found in the roots of approximately 60% of all herbaceous and tropical plants in terrestrial communities and they form an important symbiotic relationship with the plants (Scheublin et al., 2004). They grow intercellularly and intracellularly in the root cortex and form structures called arbuscules and vesicles. Arbuscules are "tree-like" structures and form between the cell wall and plasma membrane. These are the sites of metabolic exchange between the plant and the AMF (http://invam.caf.wvu.edu). Vesicles are sometimes formed inside the plant root as well. Vesicles are thin-walled lipid-containing bodies produced terminally from fungal hyphae in the root cortex (http://invam.caf.wvu.edu). There are several hyphae morphologies; they can range from absorptive to colonial to spore-bearing. The absorptive hypae absorb nutrients from the soil or sediment, colonial hyphae spread to new host plants,

and spore-bearing hyphae produce spores inside the roots as well as into the sediment in the rhizosphere (Hoefnagel, Broome, & Shafer, 1993).

Generally, AMF associations confer benefits to both plant and fungi: the fungi obtain photosynthate from the host plant and the plant benefits from added surface area for nutrient uptake (Hoefnagels et al., 1993). The increase the root surface results when AMF produce fine absorptive hyphae, which are less energetically costly to plants than producing similar sized root structures (Helgason & Fitter, 2005).

Experiments have shown that mycorrhizae are able to enhance the amounts of Nitrogen, Phosphate, Sodium, Calcium, Sulfur, Copper, and Zinc translocated from the sediment to the plant's roots (Scheublin et al., 2004). Mycorrhizae can increase nutrient uptake for the plant by increasing the absorptive surface of the roots and can decrease the stress of moving or transplanting (http://invam.caf.wvu.edu). In turn, plants provide AMF with the carbohydrates they need for their metabolic activities (Helgason & Fitter, 2005). Some studies have shown that the competitive advantages AMF provide can affect plant community competition depending on nutrient levels (Unbanhowar & McCann, 2005).

Commercial AMF products are widely available and could possibly be beneficial to marsh plants grown in a nursery setting for salt marsh restoration. Many commercial AMF products contain several species of the genera *Glomus* and *Gigaspora* such as Endomycorrhizal Innoculant (BioOrganics). *Glomus* may be utilized in commercial products because it is believed to have a fast

colonization rate (Tommerup & Abbot, 1981). In an experiment using grasses and forbs, *Glomus* isolates had colonized 92-100% of plant hosts within four weeks (Hart & Reader, 2002).

Although common commercial innoculants do not contain *G. geosporum* (the species most commonly found in salt marshes), it is expected that the inoculation of the salt marsh plant with alternative species of *Glomus* will improve the plant's growth and overall heath with no detrimental effects on the plants. A previous study by Pratt-Zossoungbo (2008) showed that *J. roemerianus* and *Schoenoplectus sp.* would successfully become colonized by AMF, whereas *S. alterniflora* was not colonized by a general commercial endomycorrhizal innoculant (Bioorganics). The innoculant used contained eight species of endospores, *Glomus aggregatum, G. clarum, G deserticola, G. intraradices, G. monosporus, G mosseae, Gigaspora margarita,* and *Paraglomus brasilianum*.

Arbuscular mycorrhizal fungi in nature and in experiments have proven to be tolerant of moderate levels of salinity in sediment (Sengupla & Chaudhuri, 1990). In salt marshes in Europe *Glomus* spores are commonly found in the sediment and 80% of the spores found are the species *G. geosporum* (Landwehr et al., 2002). Natural colonization of AMF has been demonstrated in several salt marsh plant species such as; *Puccinellia maritime* (Landwehr et al., 2002), *Distichlis stricta* (Johnson-Green, Kenkel, & Booth, 2001), and *Phragmites australis* (Dolinar & Gaberscik, 2010). All species were shown to be colonized by various species of AMF. Studies have also shown the presence of AMF spores in salt marsh sediment (Hildebrant, Karlof, & Borthe, 2001). However examined

distribution was variable in the European salt marshes (Landwehr et al., 2002). In a study of mycorrhizal colonization of *P. australis*, the freshwater and brackish collected plants were positive for AMF and endophytic fungi; and showed seasonal peaks in arbuscual formation at the end of the growing season in early September (Dolinar & Gaberscik, 2009). Field studies in salt marshes in Argentina showed that a common upper marsh species there, *Spartina densiflora*, was colonized by AMF. The same study also showed that *S. alternifora* a lower marsh species was not colonized by AMF naturally (Daleo et al., 2008). McHugh & Dighton (2004) also concluded the *S. alterniflora* was only colonized by low levels of AMF in an inoculation experiment, possibly due to its location in the lower tidal inundated salt marsh.

The presence of AMF in association with the natural occurring marsh plants of Gulf Coast has not yet been studied. But considering several salt marsh species showed positive colonization, it is likely Gulf Coast plants also have AMF associates. Although studies have shown *S. alterniflora* to be negative for AMF in North and South America (Daleo et al., 2008) there have been no specific study sites in MS. Collections of wild plants will determine if AMF naturally colonize Gulf Coast salt marsh plants. To determine if AMF shows seasonal variation, collections will be made in both fall and spring.

The purpose of the spore-trap tray experiment was to determine if the AMF that naturally colonize native salt marsh plants of the Gulf Coast can be transferred from a positively colonized plant to a negative plant through shared sediment.

The goals of the greenhouse portion this project were (a) to determine if salt marsh species in the nursery could become colonized with AFM from a commercial inoculant; (b) to determine whether the AMF would enhance the growth of the plants; and (c) to confirm that it is the inoculant fungi and not the base that enhances this growth.

CHAPTER II

MATERIALS AND METHODS

Wild-Plant Collections

The null hypothesis tested for the wild plant collections was that salt marsh plants of the Gulf Coast are not colonized by AMF with the alternative hypothesis being that that they colonized by AMF. Three each of *J. roemerianus* (hereafter referred to as *Juncus*) and *S. alterniflora* (hereafter referred to as *Spartina*) plants were collected from (between seven and 15) specific coastal salt marsh locations and an artificial beach in Ocean Springs, Mississippi USA from fall 2008 to fall 2010 (Campbell & Biber, 2009). At sites with standing water, DO, salinity, pH and temperature were recorded. The plants were collected by hand using a shovel. Roots along with sediment in the surrounding rhizosphere (about 0.25 meter down) were collected. Plant roots were placed in labeled one-gallon Ziplock bags and stored in a greenhouse. Plants were kept alive in the bags for between 2-6 weeks until their roots could be examined for AMF. It is not believed that the greenhouse plant storage would in affect the AMF colonization of wild-collected plant since they were stored in native sediment from site to reduce plant stress.

Figure 1. East Beach Dr and marshes near Gulf Coast Research Lab Ocean Springs MS, sites of wild-plant collections.

Staining Procedure

The root samples were treated using an ink and vinegar staining technique described by Verheilig et al. (1998). Selected sections of roots were rinsed thoroughly with tap water to remove sediment and debris. Rinsed roots were stored in 20 ml vials of 50% ethanol until they were ready for processing. Root tissue was then cleared with 10% KOH in a hot water bath and heated to approximately 37 degrees C for 10-20 minutes (depending on plant species). Cleared roots were rinsed in tap water and stained with a 5% Schaeffer black ink and vinegar stain in the same heated water bath for 10-20 minutes (depending on species), rinsed with tap water, and stored in acidified tap water (Vierheilig et al., 1998).

Using this method the chitinous structures of the AMF in root tissues were stained dark blue (Figure 1). All stained roots were mounted on microslides and

reviewed under a microscope at 4x magnification. Roots that were not colonized by AMF remain transparent (Figure 2). There are several methods for quantifying AMF colonization, but for this experiment, presence or absence of AMF was the only variable measured.

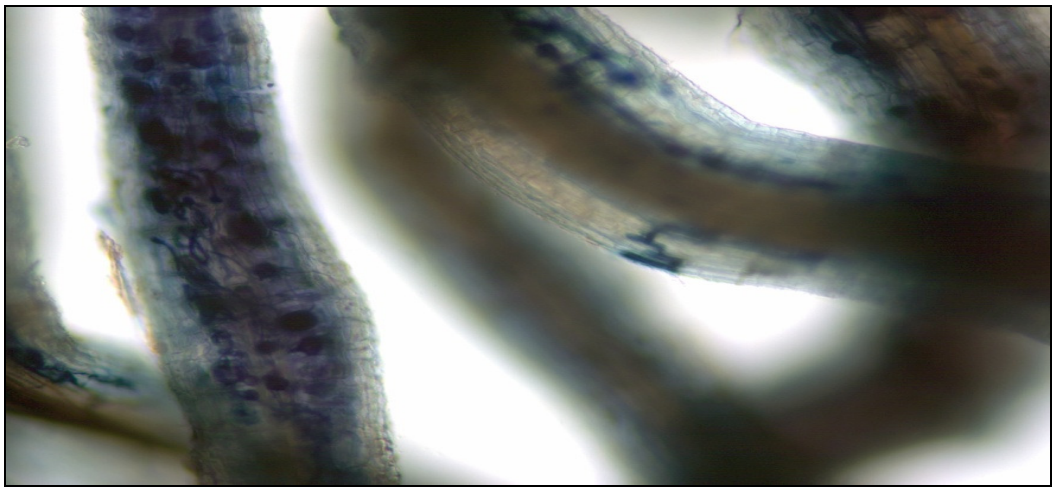

Figure 2. AMF colonized root, dark blue stained hyphae, vesicles, and arbscules, viewed at 4x magnification.

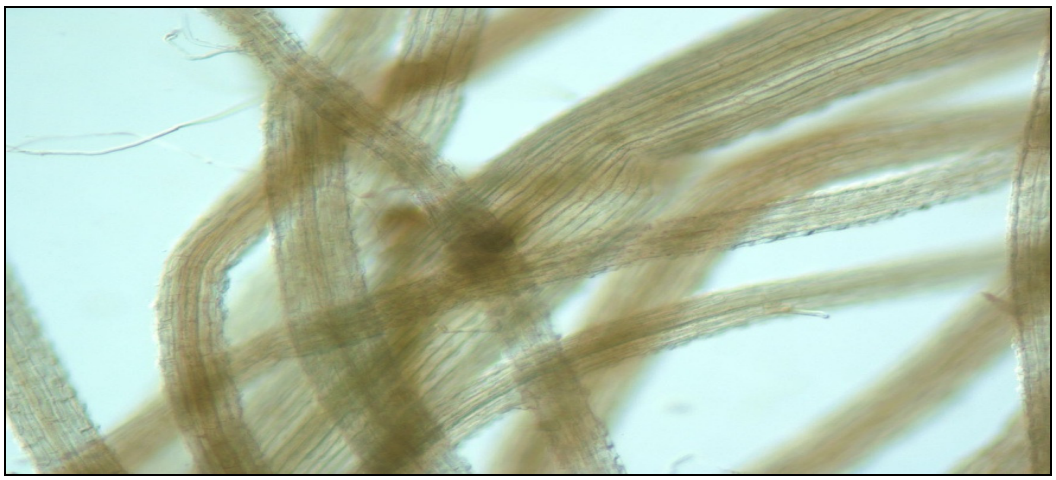

Figure 3. Clear root, uncolonized by AMF viewed at 4x magnification.

Spore-Trap Tray Set-Up

The null hypothesis tested by the spore-trap trays was that the AMF

colonization would not transfer to the plants negative for colonization through shared sediment with the alternative hypothesis that the colonization would successfully spread from the AMF positive plants to the negative ones. Spore-trap trays were used to determine if the natural AMF will transfer from a colonized plant to a non-colonized plant of the same species through shared sediment. Spore-trap trays have been developed for use in agriculture to promote the spread of beneficial AMF from colonized to un-colonized plants.

Three 0.3 x 0.5 m plastic trays were set up in a greenhouse. Each tray contained six wild-collected *Spartina*, three of which were colonized (marked with blue bands) by AMF, interspersed with three wild-collected un-colonized plants (marked with yellow bands). The plants were placed in the tray with sediment from the wild-plant collection site in a 50:50 peat/sand mixture in April of 2009. The plants were potted in an alternating colonized and un-colonized configuration to promote AMF colonization. The roots of the plants were examined nine months later in December to determine whether plants in the tray maintained their colonization and whether the AMF spread to the un-colonized *Spartina* plants.

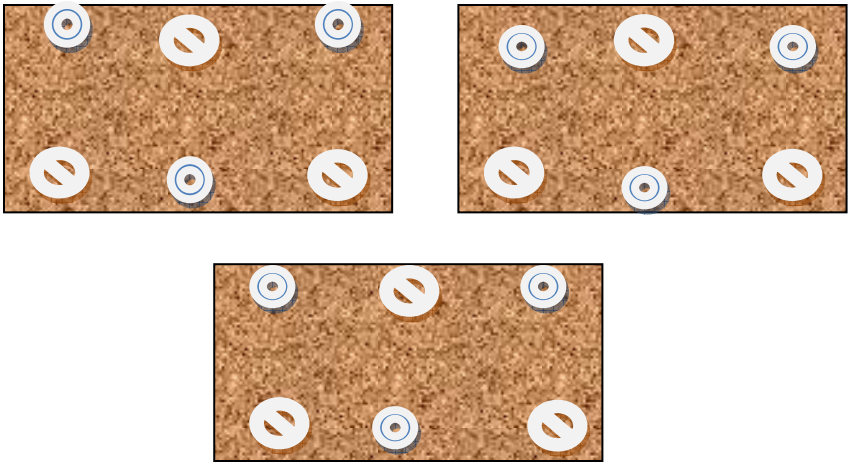

Figure 4. Spore-tray set-up. Each square is a tray, each blue open circle is a *Spartina* plant colonized with AMF and each yellow slashed circle is an un-colonized *Spartina* plant.

Greenhouse Experimental Design

The null hypothesis tested by the greenhouse experiment was that the commercial agricultural AMF inoculant would not cause AMF colonization of salt marsh plants and effect plant growth, with the alternative hypothesis that the colonization would be obtained and positively affect the plants' growth. Three experiments were performed using 17 mature *Juncus,* 12 *Spartina,* and 15 *S. americanus* plants (hereafter referred to as *Schoenoplectus*). The plants were nursery-raised at the Gulf Coast Research Lab (GCRL) or wild-collected and found to be negative for AMF prior to beginning the experiment. The nursery-raised plants were used preferentially if found to be negative for AMF. When there were not enough nursery plants wild-collect plants were used. All plants were maintained inside a greenhouse for the duration of the experiment. Approximately once every two months all plants in the greenhouse received a dilute nutrient solution (Miracle Grow, 20N:20P:20K).

A commercial inoculant, Biogrow Hydro-sol (Holland's Land O'Giants), was used as the treatment. This inoculant contains four species of AMF of the genus *Glomus*: *G. intraradices, G. mosseae, G. aggregatum,* and *G. etunicatum*. Four tablespoons of Hydro-sol were mixed with one liter of tap water to make the inoculant treatment solution as per manufacture's instructions. The base solution used was made from four tablespoons of the medium for the Biogrow Hydro-sol containing inert matter, but without any fungal spores.

The *Juncus* and *Schenoplectus* were planted in four-inch square pots filled with 50:50 ratio of top-soil to sand, and each species divided into three groups of five to six plants: control, base and treatment. Individual plants in the control, base, and treatment groups were separated from each other to avoid unwanted contamination of AMF treatment in the control and base trays. Each of the larger trays was labeled, and only contained plants from one of the groups. The *Juncus* experiment was started on 1 September 2009, for which trays were designated control (JC), base (JB) or treatment (JT). The *Schoenoplectus* experiment was started on 11 December 2009, and for which trays were designated control (ScC), base (ScB) and treatment (ScT). The *Spartina* experiment also started on 11 December 2009 and comprised only two groups, designated control (SC) and treatment (ST). Plants representing each treatment group were placed within single trays to maintain similar ambient light and temperature for all plants in the greenhouse experiments, while also utilizing limited shelf space. All the trays were placed on the same shelf in the greenhouse and lighting appeared to be fairly uniform for the entire shelf.

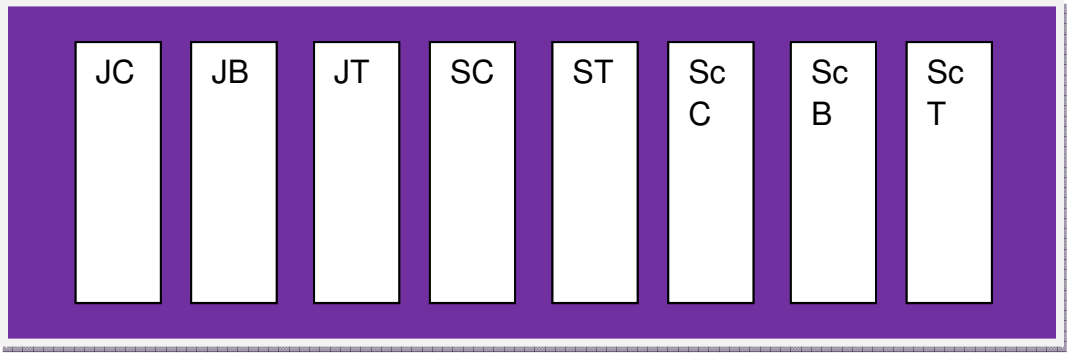

Figure 5. Greenhouse experiment plant set-up, larger purple rectangle is the greenhouse shelf and each white rectangle is one tray of plants.

Trays JT, ScT, and ST were allocated 10 ml of inoculant weekly; trays JB and ScB were given 10 ml of inoculant base weekly; trays JB, ScC, and SC were given 10 ml of water. All three groups were watered to saturation daily with fresh water from above so that the soil was soaked from the top down. Heights of all plants were measured once weekly and the number of shoots was counted. For the SC and ST trays, leaves were also counted weekly.

Root Collection and Mycorrhizal Detection

For mycorrhizal detection, samples were taken from three different zones from inside and outside the root ball of the experimental plants. At first, some roots were removed and stained from one of the plants in the JC tray and all of the plants from the JB and JT trays on a monthly basis. The monthly root removal and staining took place for three consecutive months. Because this process negatively affected the plants by eliciting signs of stress (e.g., shoot loss, slowed growth), the root removal and staining process was postponed until the end of the experiment. Root samples were stained using the ink-vinegar technique described previously.

Above and below-ground dry-weight biomass of each plant was separately

measured at the end of the experiment. Roots and shoots were carefully cleared of the soil mixture using tapwater and tweezers. After the roots were allowed to air-dry, both the above and below-ground sections of the plant were weighed. Then the sections were dried in an oven at 40 degrees Celsius for three days and weighed to 0.001 mg accuracy.

Data Analysis

The seasonal results of wild-collected plants were examined using a chi square analysis to determine if AMF colonization was dependent or independent of season (Zar, 1999). The null hypothesis was that the AMF colonization of plant was independent of season. The alternate hypothesis was that AMF colonization was dependent on the season. The spore-trap plants were not subjected to statistical analysis since presence or absence of AMF was the only parameter recorded.

Plant growth in terms of shoot number and shoot height for the greenhouse experiments were analyzed using Univariate Repeated-Measures ANOVA (RM ANOVA) (Green & Salkind, 2000). The within-subject factor (TIME) for the RM ANOVAs accounts for non-independence of serial measurements of the same subjects across time. Weekly measurement data for the RM ANOVA's were pooled into 6-week intervals to stabilize growth signals and decrease the necessary degrees of freedom taken up by the analyses. Separate RM ANOVA analyses were conducted for each response by each species of emergent vegetation. The between-subjects factor (TYPE) of the RM ANOVA's comprised three levels for *Juncus* and *Schoenoplectus*: Control, Base, and Treatment;

whereas the between-subjects portion for the *Spartina* responses only comprised Control and Treatment levels. The RM ANOVA model tested for the interaction between TIME and TYPE, as well as for the main effects associated with these two factors. Greenhouse-Geisser corrected test values were used to assess significance of the RM ANOVA's in the face of any lack of conformity to the sphericity assumption. Tests of Within-Subjects effects examined whether trends in time fit linear or polynomial relationships. Differences among levels were assessed using Least Significance Difference tests for analyses involving more than two between-subject levels. Homogeneity of variance among levels of the between-subjects factor (TYPE) were tested at each level of the within-subjects factor (TIME) using Levene's tests of the equality of error variances. Differences in final dry-weight biomass in the greenhouse experiment were analyzed separately for each plant species using a One-Way ANOVA (Zar, 1999).

CHAPTER III

RESULTS

Wild-Plant Collections

Collections began in the fall of 2008 and were continued twice annually until fall of 2010. At every site 3 *Juncus* and 3 *Spartina* were collected (6 plants total). The first collections in the fall of 2008 (Campbell & Biber, 2009) showed that *Juncus* and *Spartina* were both colonized by AMF, with 64% of the collection sites exhibiting at least one of the six plants collected colonized by AMF. By the spring of 2009 (Campbell & Biber, 2009), 100% of the sites had at least one positive plant. In the fall of 2009, 57% of sites had at least one positive plant. In the spring of 2010, 100% of the sites had at least one colonized plant. In the fall of 2010, 67% of the sites had at least one colonized plant. Together, these findings indicate a seasonal change in colonization by AMF which is higher in the spring (100%) and lower in the fall (57-67%) (Table 1).

Table 1

AMF Colonization of Salt Marsh Seasonal Collection Sites in Percent

Season	Colonized Sites	Uncolonized Sites
Fall 2008	64%	36%
Spring 2009	100%	0%
Fall 2009	57%	43%
Spring 2010	100%	0%
Fall 2010	67%	33%

Spore-traps

The three *Spartina* spore-traps were set up in April 2009 and the trays were watered daily with fresh water. The trays were allowed to grow for period of nine months undisturbed to give the AMF spores adequate time for transfer of colonization. The plants within the trays grown until December in the greenhouse at which point the roots were examined at 4x magnification in January. In spore-trap tray 1, all three of the previously colonized *Spartina* plants retained evidence of AMF colonization and all three plants which were initially negative for colonization were also found to be positive for AMF colonization. In spore-trap tray 2, all three the previously colonized *Spartina* plants retained evidence of AMF colonization and two of the three plants which were initially negative for

colonization were also found to be positive for AMF colonization In spore-trap tray 3, the three previously colonized *Spartina* plants retained evidence of AMF colonization, and two of the three plants which were initially negative for colonization were also found to be positive for AMF colonization. In spore-trap tray 3, the three previously colonized *Spartina* plants retained evidence of AMF colonization, and two of the three plants which were initially negative for colonization were also found to be positive for AMF colonization (Table 2).

Table 2

Summary of Colonization Percent of Un-Colonized Plants in Each Spore-Tray

Spore-trap trays	
Tray number	Percent of colonized previously un-colonized plants
1	100%
2	33%
3	67%

Greenhouse Experiments - Plant Growth

The greenhouse experiments were started on two dates; The *Juncus* control, base, and inoculant groups were all planted in the four inch experiment pots on September 1, 2009. The *Spartina* control and inoculant groups and *Schoenoplectus* control, base, and inoculant groups were planted in four-inch

experiment pots on December 11, 2009. After up to a year of weekly measurements of height, as well as shoot and leaf counts, the data was pooled into 6 week intervals as described above.

The *Juncus* plants in all trays started out with an average height between 20 and 25 cm. At the end of the experiment all the *Juncus* plants in all trays reached a mean height ranging from 30 to 40 cm, with the plants in the treatment tray (JT) averaging slightly greater in height than plants in control and base trays (JC and JB). After only modest growth, a marked growth spurt for plants in all three groups occurred between weeks 30 and 36, after which growth diverged somewhat among the three groups (Figure 6).

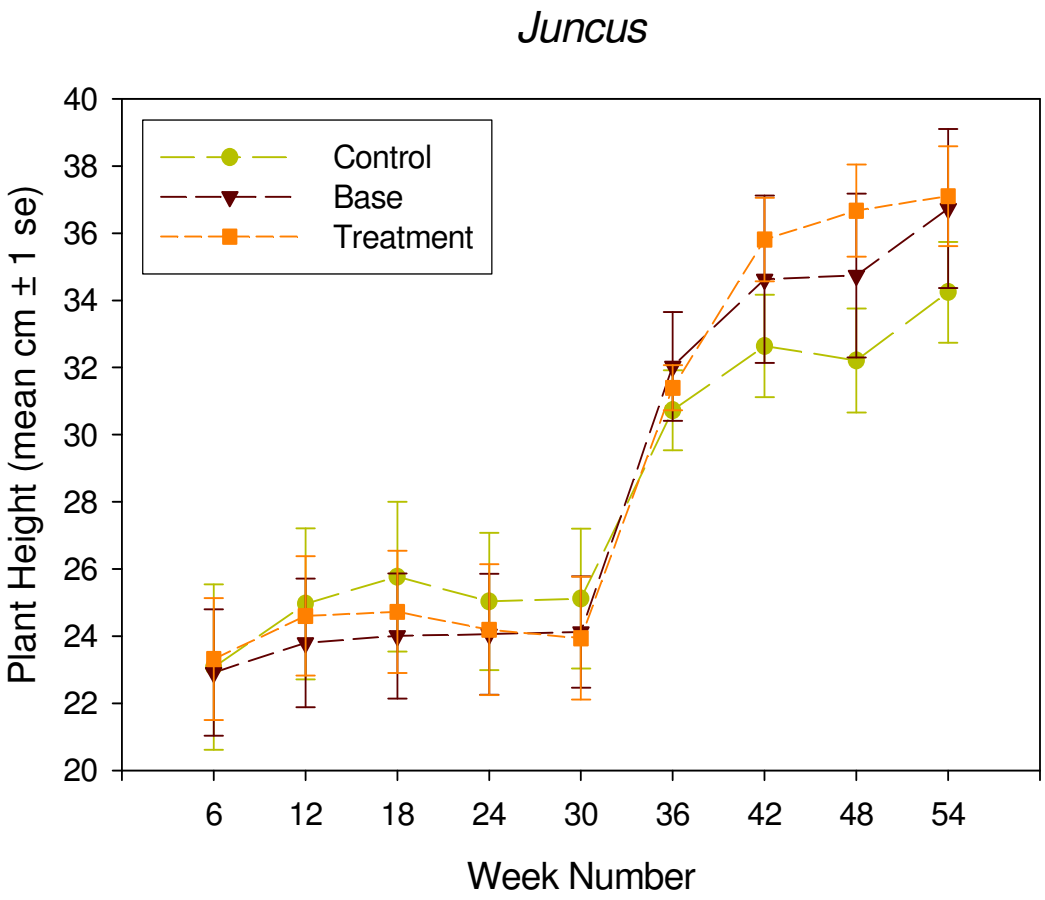

Figure 6. Average (± 1 se) Change in Height Over 6-Week Periods for Trays JC (n=5), JB (n=6) and JT (n=6).

The *Juncus* plants in all trays started out with shoot counts averaging between 50 and 100 per pot with slightly more shoots in the JT tray. At the end of the experiment all the *Juncus* plants in all trays exhibited a mean shoot count ranging from 100 to 150. Plants in the tray JC averaged slightly higher in number than plants in trays JB and JT. Shoot number changed across time in a parallel fashion for all three groups throughout the experiment (Figure 7).

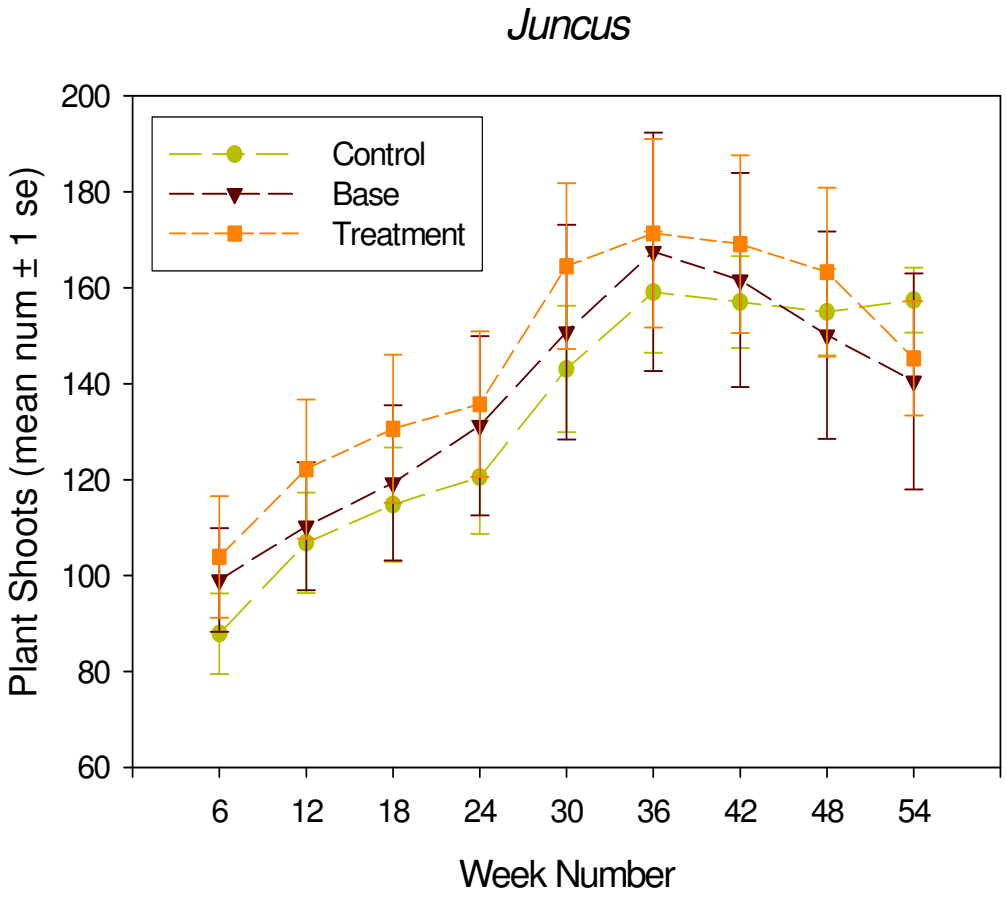

Figure 7. Average (± 1 se) Change in Shoot Number Over 6-Week Periods for Trays JC (n=5), JB (n=6) and JT (n=6).

The *Schoenoplectus* plants in all trays started out with a mean height between 20 and 40 cm. By the end of the experiment, the height of all the *Schoenoplectus* plants averaged from 60 to 80 cm across all trays, but the plants

in the tray ScT averaged lower in height than plants in trays ScC and ScB. The mean height for the treatment group was notably lower than the other groups between weeks 12 and 30 (Figure 8).

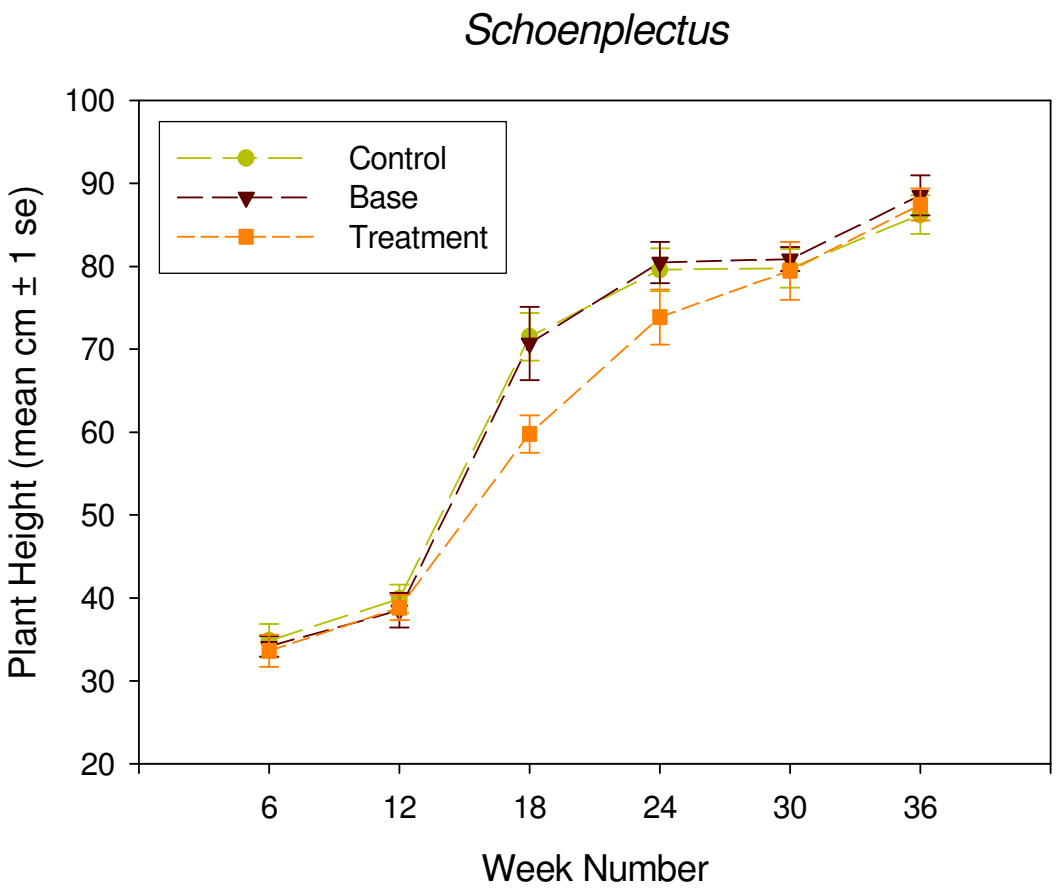

Figure 8. Average (± 1 SE) Change in Mean Height Over 6-Week Periods for Trays ScC (n=5), ScB (n=5), and ScT.

The *Schoenoplectus* plants in all trays started out with a mean shoot count between one and five per pot. At the end of the experiment all the *Schoenoplectus* plants in all trays exhibited a mean shoot count ranging from 10 to 15. Plants in the tray ScC averaged slightly higher in shoot counts than plants in trays ScB and ScT from week 24 to week 36 (Figure 9).

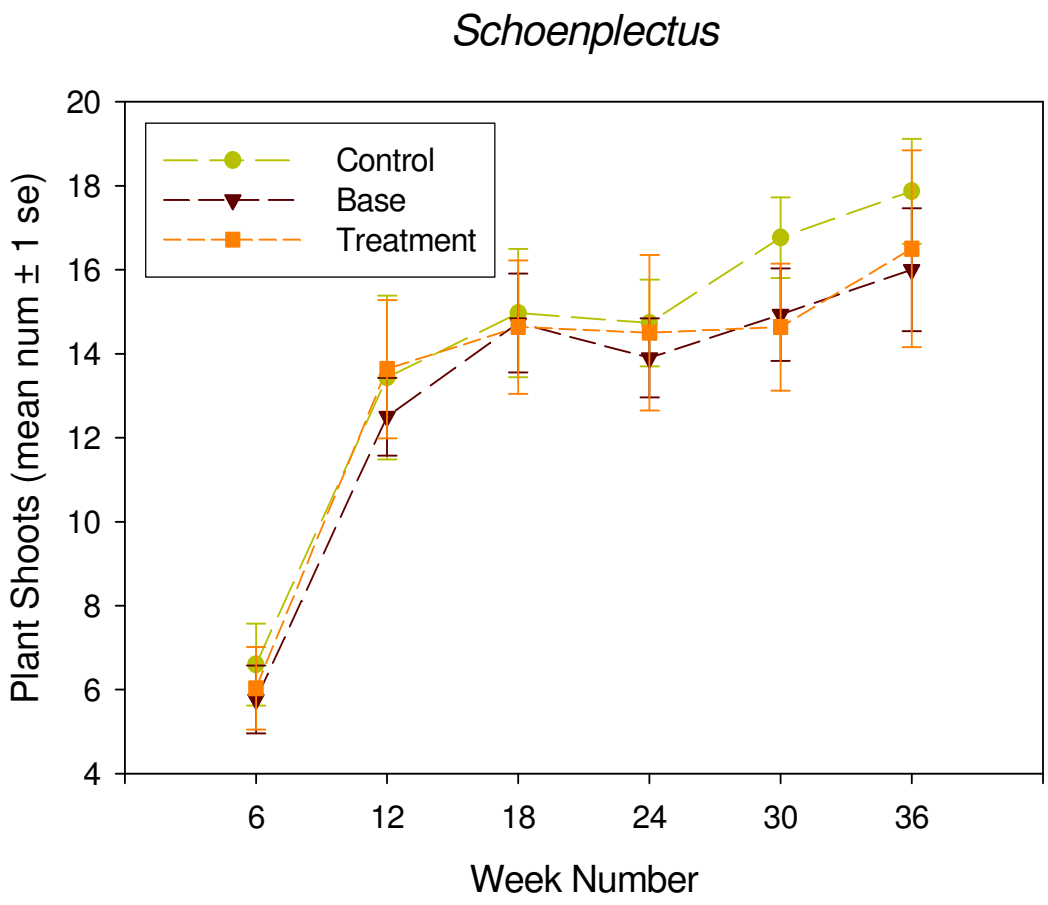

Figure 9. Average (± 1 SE) Change in Mean Shoot Number Over 6-Week Periods for Trays ScC (n=5), ScB (n=5) and ScT (n=5).

The *Spartina* plants in all trays started out with a mean height between 20 and 40 cm, with the plants in tray SC exhibiting a higher mean height. At the end of the experiment the *Spartina* plants in all trays showed a mean height ranging from 40 to 60 cm, and the plants in the tray ST were higher height than plants in trays SC (one plant in tray SC died in mid-experiment). Possibly due to the death of one plant, the standard error in the SC tray was especially high during the last half of the experiment (Figure 10).

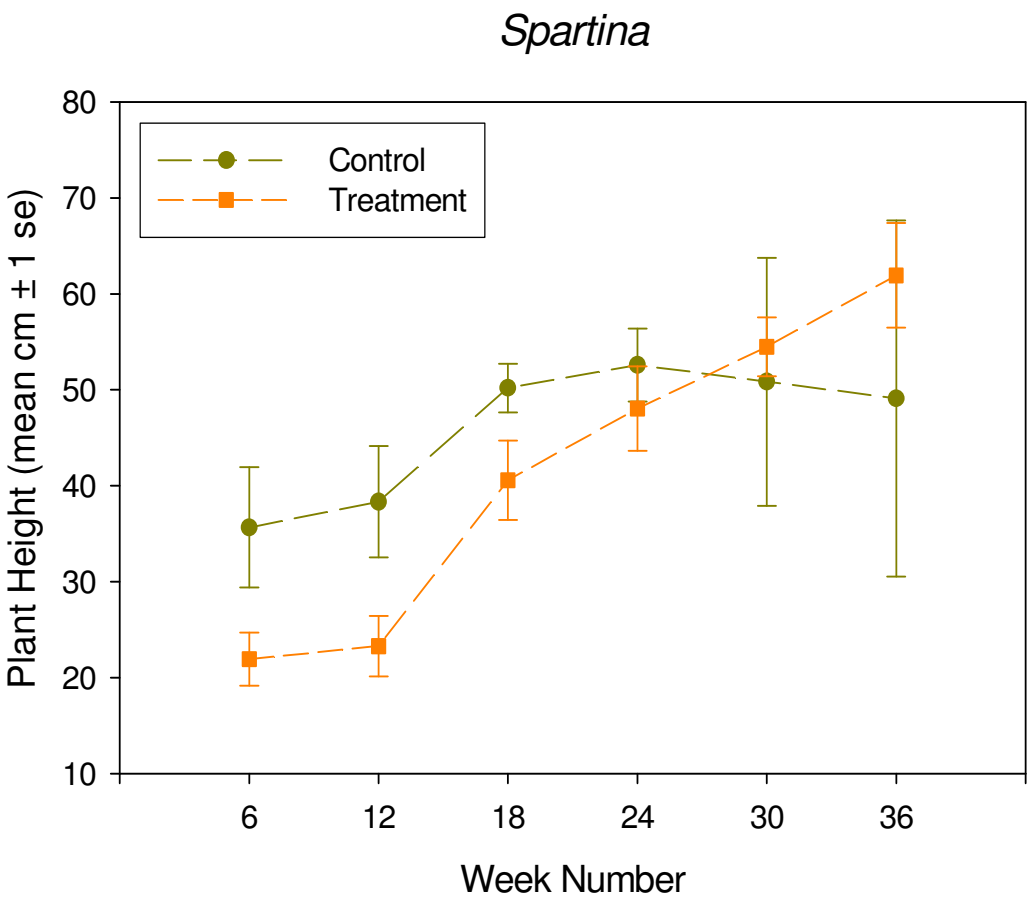

Figure 10. Average (± 1 SE) Change in Mean Height Over 6-Week Periods for Trays SC (n=6) and ST (n=6).

The *Spartina* plants in all trays started out with a mean shoot count between one and two shoots per pot. At the end of the experiment all the *Spartina* plants in all trays showed a mean shoot count ranging from four to six, and plant shoot counts in the SC tray averaged higher while varying increasingly over time relative to plants in ST trays (Figure 11).

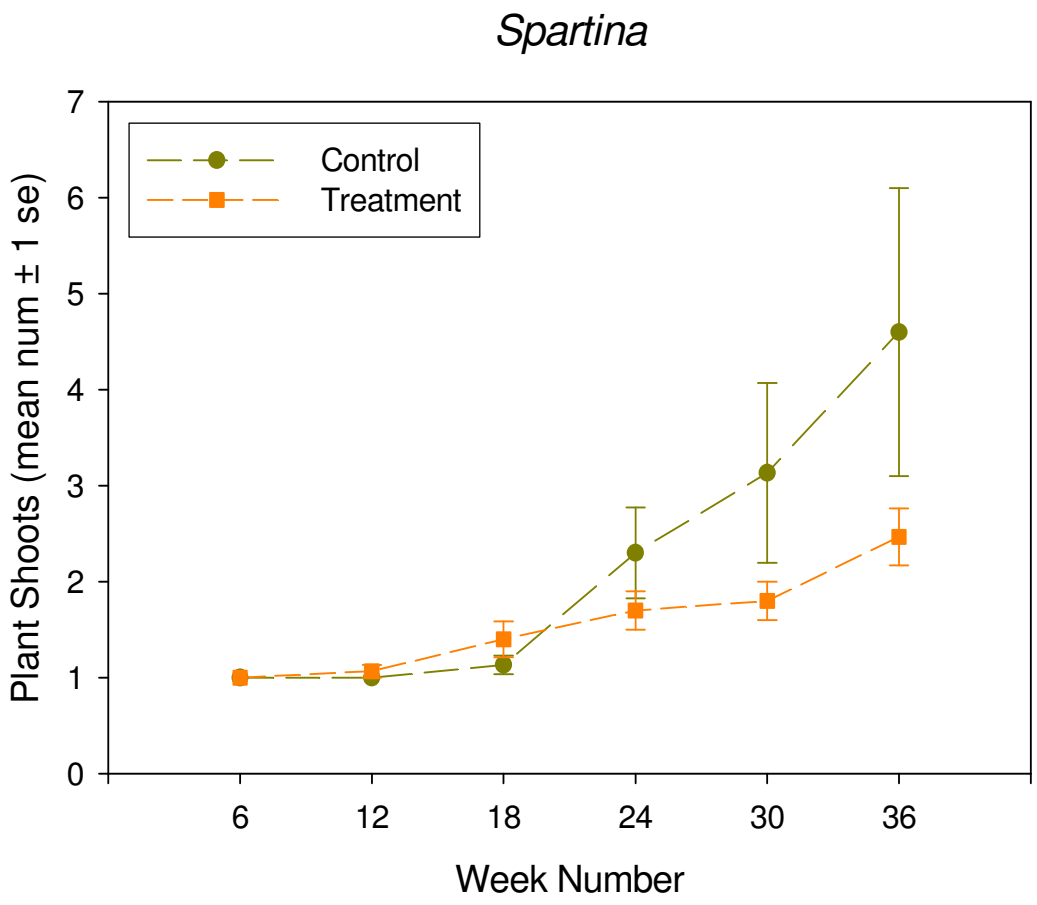

Figure 11. Average (± 1 SE) Change in Mean Shoot Number Over 6-Week Periods for Trays SC (n=6) and ST (n=6).

Spartina plants in all trays started out with a mean leaf count between five and seven leaves per pot. The leaf count peaked at week 18 for both groups, and then declined. There was a great deal of variation in leaf number in control plants during the latter half of the experiment. However, the means for both *Spartina* groups were comparable at the end of the experiment, when the mean leaf count ranged from five to seven (Figure 12).

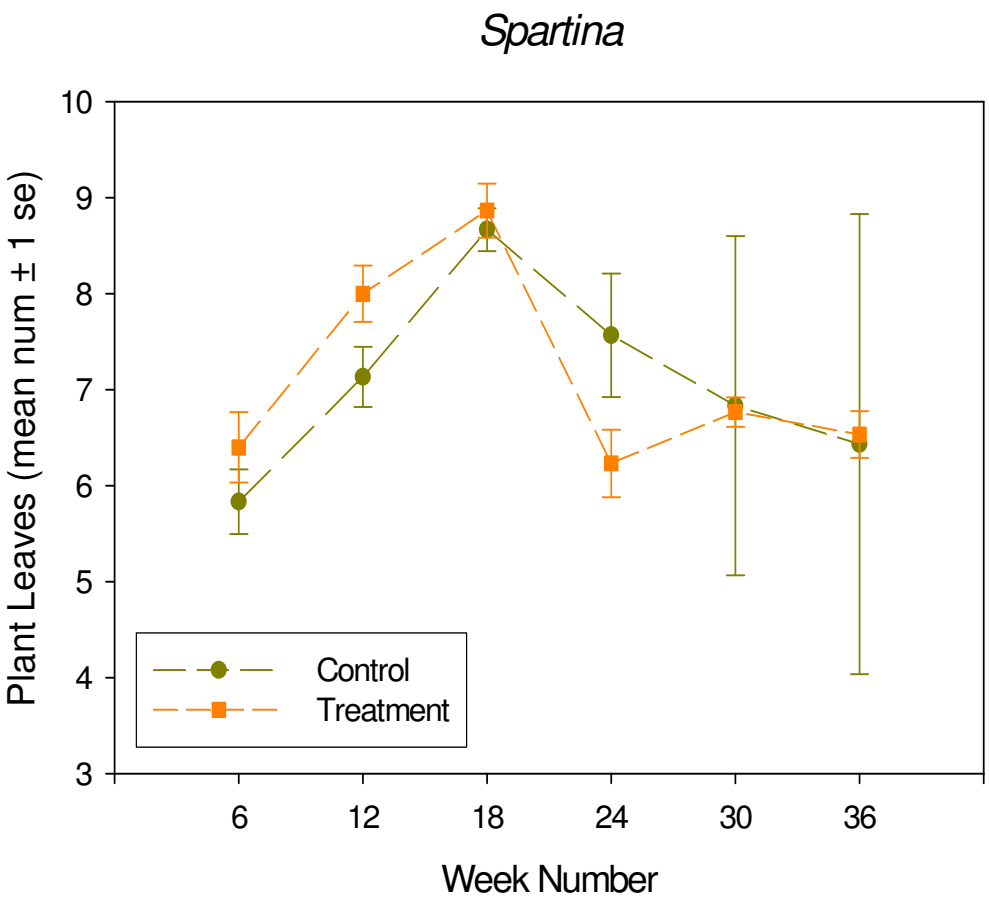

Figure 12. Average (± 1 SE) Change in Mean Leaf Number Over 6-Week Periods for Trays SC (n=6) and ST (n=6).

In addition to the weekly shoot counts and height measurements, above ground and below ground dry-weight biomass was measured for every plant at the end of the experiment. The *Juncus* plants in tray JC had a mean total dry-weight of 17.463 grams. The mean above ground dry-weight for plants in tray JC was 7.387 grams and the mean below ground biomass was 10.077 grams. The mean above ground/total dry weight for the plants in JC was 42.3 and the mean below ground/total dry weight was 57.7. The *Juncus* plants in tray JB had a mean total dry-weight of 14.120 grams. The average above ground dry-weight for plants in tray JB was 6.693 grams and average below ground biomass was 7.482 grams. The average ratio of above ground/total dry weight for the plants in JB was 47 and

the average ratio of below ground/total dry weight was 53. The *Juncus* plants in tray JT had a mean total dry-weight of 13.695 grams. The mean above ground dry-weight for plants in tray JT was 5.528 grams and the mean below ground biomass was 8.167 grams. The mean ratio of above ground/total dry weight for the plants in JT was 40.4 and the mean ratio of below ground/total dry weight was 59.6.

The above ground and below ground dry weight biomass means for the *Juncus* plants in trays JC, JB, and JT were compared. The plants in JC had both higher above ground (Figure 13) and below ground dry weight biomass means (Figure 14).

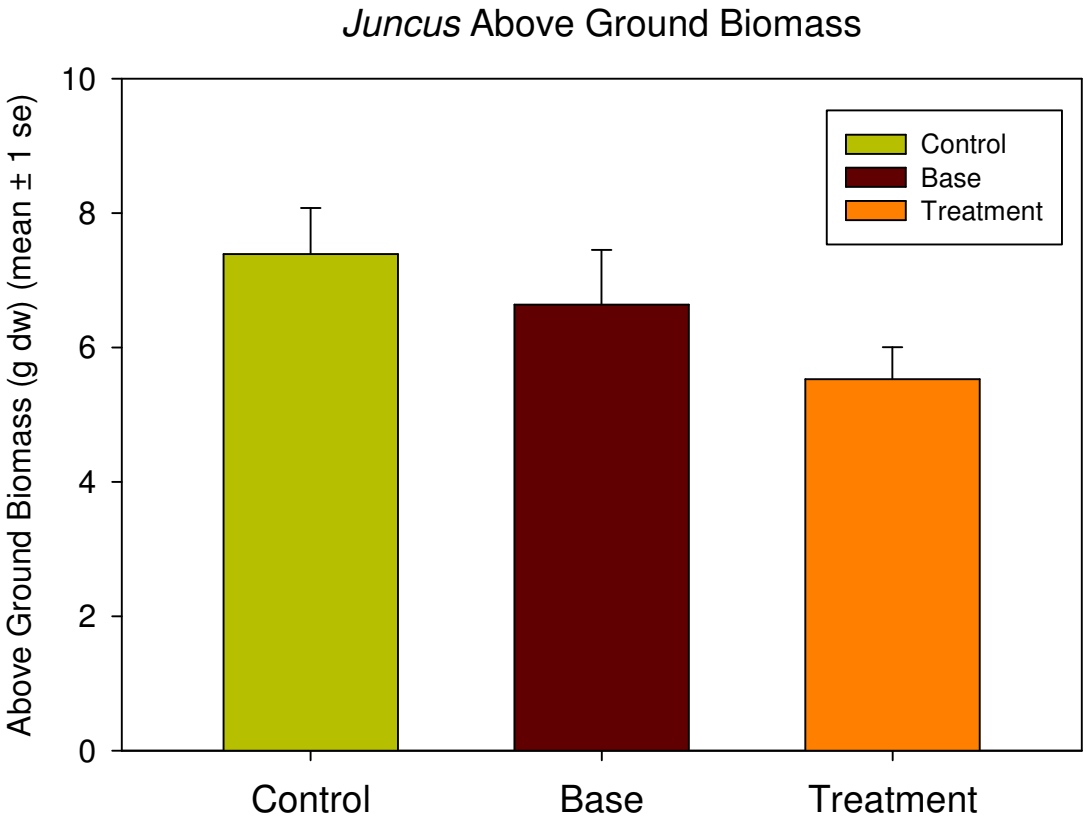

Figure 13. Mean (±1 SE) Above Ground Dry Weight Biomass of *Juncus* Plants in trays JC (n=5), JB (n=6), and JT (n=6).

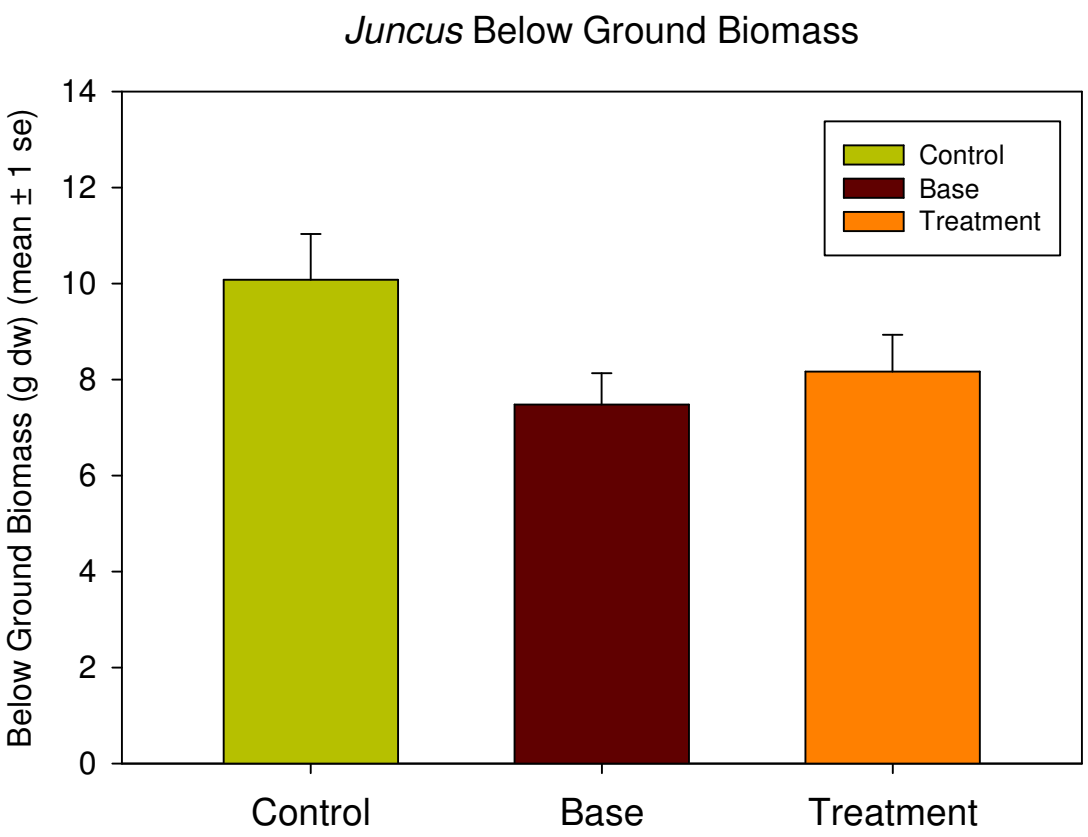

Figure 14. Mean (±1 se) Below Ground Dry Weight Biomass of *Juncus* Plants in Trays JC (n=5), JB (n=6), and JT (n=6).

The *Schoenoplectus* plants in tray ScC had an mean total dry-weight of 20.327 grams. The mean above ground dry-weight for plants in tray ScC was 4.853 grams and the mean below ground biomass was 15.474 grams. The mean ratio of above ground/total dry weight for the plants in ScC was 23.9 and the average ratio of below ground/total dry weight was 76.1. The *Schoenoplectus* plants in tray ScB had a mean total dry-weight of 18.530 grams. The mean above ground dry-weight for plants in tray ScB was 4.723 grams and the mean below ground biomass was 13.807 grams. The mean ratio of above ground/total dry weight for the plants in ScB was 25.5 and the mean ratio of below ground/total dry weight was 74.5. The *Schoenoplectus* plants in tray ScT had a mean total

dry-weight of 19.072 grams. The mean above ground dry-weight for plants in tray ScT was 4.224 grams and the mean below ground biomass was 14.848 grams. The mean ratio of above ground/total dry weight for the plants in ScT was 22.1 and the mean ratio of below ground/total dry weight was 77.9.

Above ground and below ground dry weight biomass means for the *Schoenoplectus* plants in trays ScC, ScB, and ScT were compared. The plants in ScC had both higher above ground (Figure 15) and below ground dry weight biomass means (Figure 16).

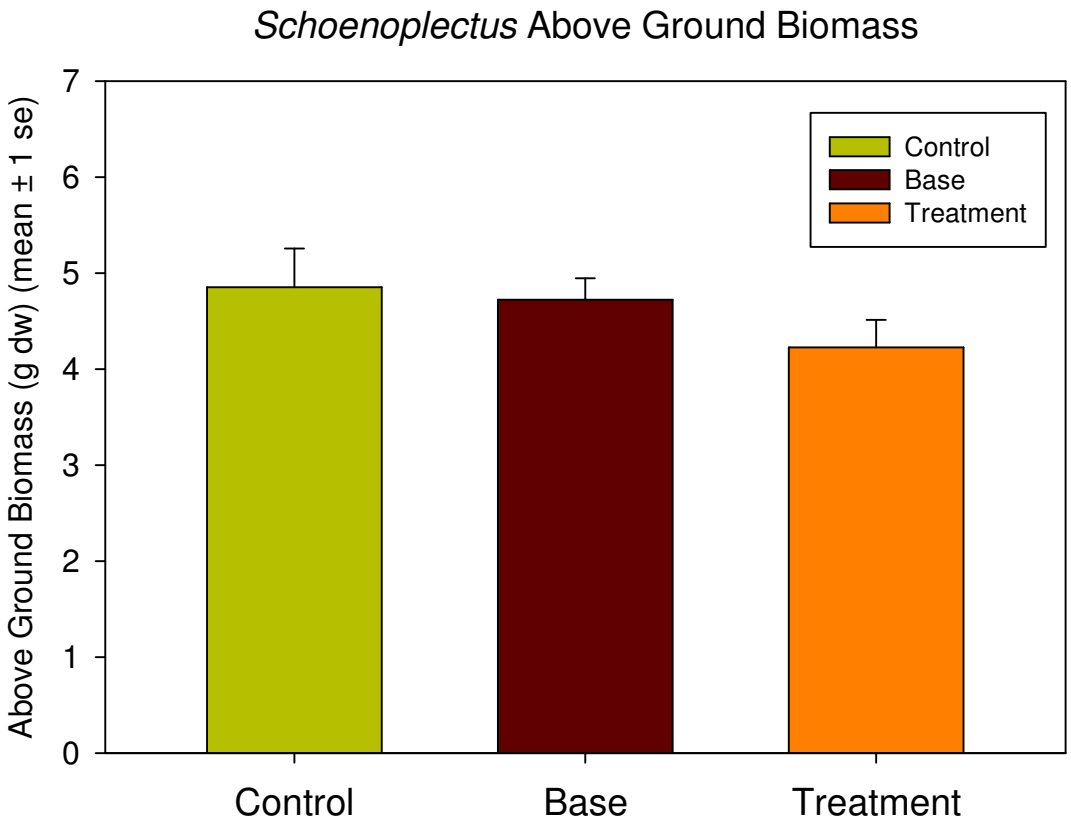

Figure 15. Mean (±1 SE) Above Ground Dry Weight Biomass of *Schoenoplectus* Plants in Trays ScC (n=5), ScB (n=5), and ScT (n=5).

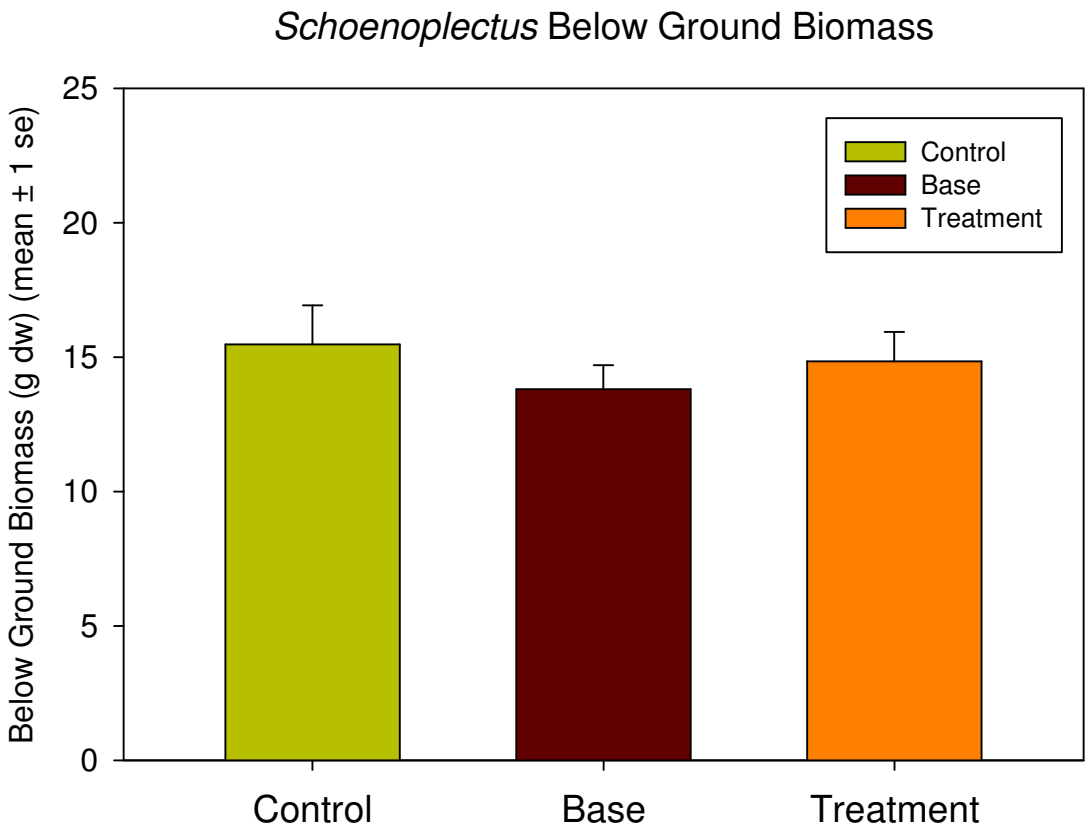

Figure 16. Mean (±1 se) Below Ground Dry Weight Biomass of *Schoenoplectus* Plants in Trays ScC (n=5), ScB (n=5), and ScT (n=5).

The *Spartina* plants in tray SC had a mean total dry-weight of 10.824 grams. The mean above ground dry-weight for plants in tray SC was 4.447 grams and the mean below ground biomass was 6.377 grams. The mean ratio of above ground/total dry weight for the plants in tray SC was 41.1 and the mean ratio of below ground/total dry weight was 58.9. One plant in tray SC died before any measurements could be taken. The *Spartina* plants in tray ST had a mean total dry-weight of 8.791 grams. The mean above ground dry-weight for plants in tray SC was 3.601 grams and the mean below ground biomass was 5.190

grams. The mean ratio of above ground/total dry weight for the plants in tray SC was 41.0 and the mean ratio of below ground/total dry weight was 59.0.

The above ground and below ground dry weight biomass means for the *Spartina* plants in trays SC and ST were compared. The plants in SC had both higher above ground (Figure 17) and below ground dry weight biomass means than ST (Figure 18).

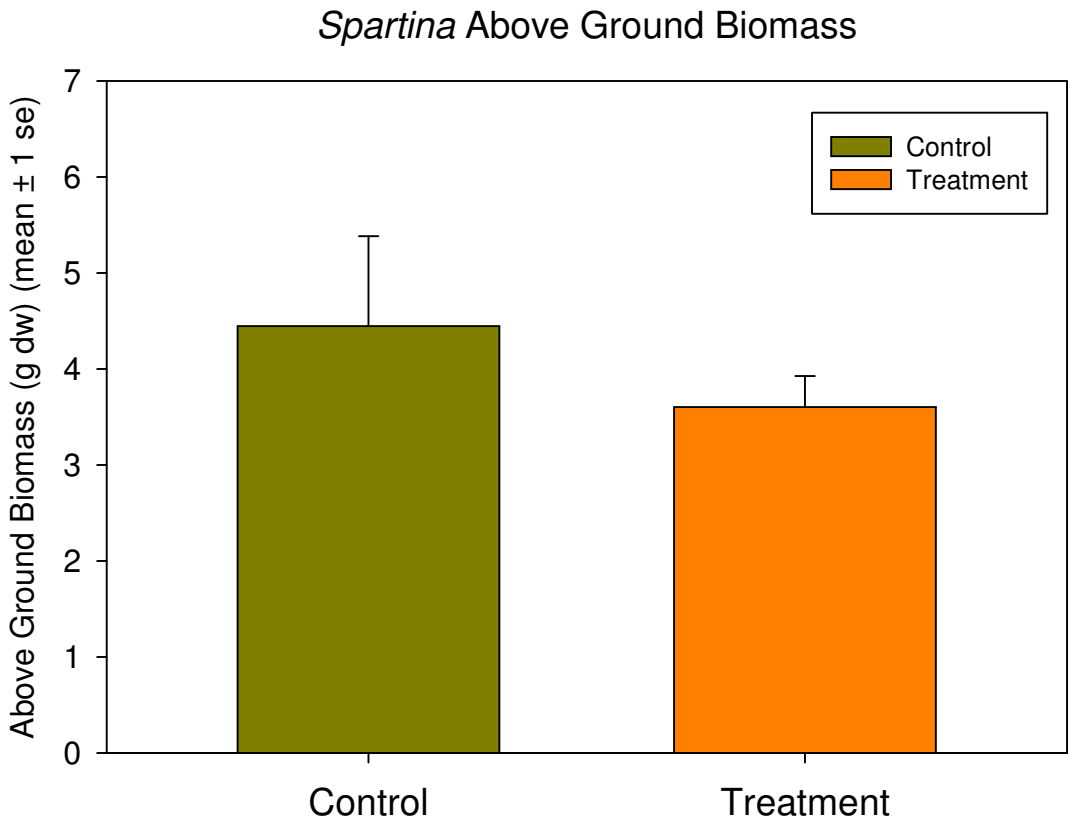

Figure 17. Mean (±1 SE) Above Ground Dry Weight Biomass of *Spartina* Plants in Trays SC (n=6) and ST (n=6).

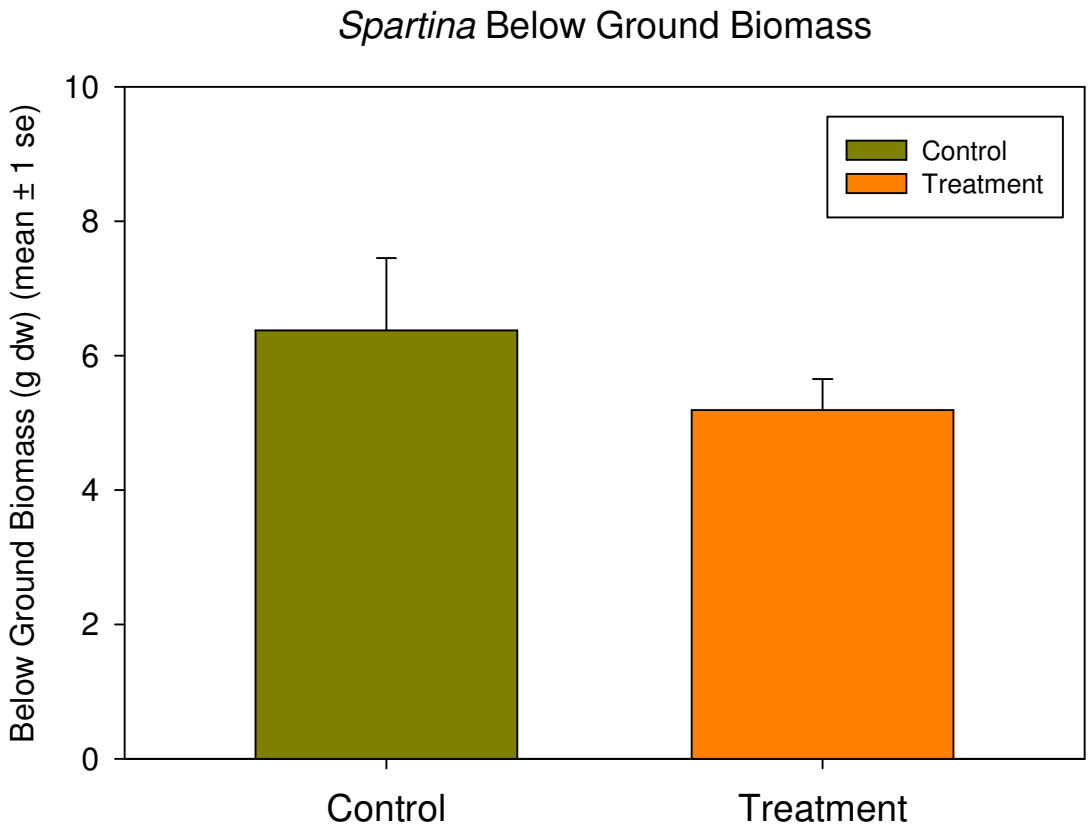

Figure 18. Mean (±1 SE) Below Ground Dry Weight Biomass of *Spartina* Plants in Trays SC (n=6) and ST (n=6).

After 12 months of growth for the *Juncus* experiment plants and 9 months for the *Schoenplectus* and *Spartina* all the plants were examined for AMF colonization using the root staining technique described previously. The *Juncus* plants in trays JC and JB were all negative for AMF colonization. Two of the six *Juncus* plants in tray JT were colonized by AMF at very low levels, for a colonization rate of 33% (Table 3) The *Schoenoplectus* plants in trays ScC and ScB were all negative for AMF colonization. One of the five *Schoenoplectus* plants in tray ScT were colonized by AMF, for a colonization success of 20%.The

Spartina plants in trays SC were negative for AMF colonization. Two of the six *Spartina* plants in tray ST were colonized by AMF, for a colonization success of 33%.

Table 3

Colonization of Treatment Groups JT (n=5), ScT (n=5), ST (n=6) in Greenhouse Inoculant Experiments

Colonization % of treatment groups	
JT	33%
ScT	20%
ST	33%

Data Analysis

The seasonal pattern of AMF colonization in wild-collected plants was examined using a chi square test to determine if colonization was independent of season. The critical chi-square value at $\alpha = 0.05$, df =1 (1 degree of freedom) was 3.841 (Zar, 1999). The resulting chi-square value of 7.770 for the seasonal pattern was greater than the critical value the null hypothesis; thus, the hypothesis of seasonally independent AMF colonization was rejected, and the alternative hypothesis that AMF colonization is dependent on season was accepted.

The growth rate variables for the greenhouse experiment plants (height/time, shoot growth/time and leaf count/time) were analyzed using

univariate Repeated-Measures ANOVA for the greenhouse experiment. Levels of the within-subject factor (TIME) were defined by 6-week intervals and levels of the between-subjects factor (TYPE) were defined by the control, base, and treatment groups. TYPE was not tested alone since the measurements were repeated over time. Each growth rate variable was analyzed separately for each plant species. Within-subject effects were checked for sphericity assumption. Sphericity is an inherent assumption of repeated measures ANVOA (Zar, 1999). When sphericity assumption was not met Greenhouse-Geisser tests (an approximation procedure) were used to interpret the results.

The *Juncus* plants in tray JT had a mean height of 29.083 cm with a standard deviation of 1.511cm which was slightly higher than the plants in trays JC (28.197± 1.655) and JB (28.558 ± 1.511) The Greenhouse-Geisser test showed that TIME was significant and that the TIME*TYPE interaction was non-significant (Table 4). Within-Subjects Contrasts showed that there was a linear trend over time. The Between-Subjects test failed to show an overall significant difference among groups for the TYPE factor (P = 0.923) (Table 5).

Table 4

Tests of Within-Subjects Effects for Juncus *Height Measurements/Time*

Tests of Within-Subjects Effects for *Juncus* height/time						
Source		Type III Sum of Squares	df	Mean Square	F	Significance
Time	Sphericity Assumed	3844.897	8	480.612	<0.001	<0.001
	Greenhouse-Geisser	3844.897	1.333	2883.497	<0.001	<0.001
Time * Type	Sphericity Assumed	112.683	16	7.043	0.388	0.388
	Greenhouse-Geisser	112.683	2.667	42.253	0.378	0.378

Table 5

Tests of Between-Subjects Effects for Juncus *Height Measurements/Time*

Tests of Between-Subjects Effects					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	19.787	2	9.894	0.080	0.923
Error	1725.046	14	123.218		

The *Juncus* plants in tray JT had a mean count of 145.07 shoots per pot with a standard deviation of 15.431, which was slightly higher than the plants in

trays JC (133.49 ± 16.904) and JB (146.69 ± 15.43). The Greenhouse-Geisser test showed that TIME was significant and that the TIME*TYPE interaction was non-significant (Table 6). Within-Subjects Contrasts showed that there was a linear trend over time. The Between-Subjects test failed to show an overall significant difference among groups for the TYPE factor (P = 0.870) (Table 7).

Table 6

Significance Levels for Tests of Within-Subjects Effects for Juncus *Shoot Counts/Time*

Tests of Within-Subjects Effects for *Juncus* shoot count/time

Source		Type III Sum of Squares	df	Mean Square	F	Significance
Time	Sphericity Assumed	78875.635	8	9859.454	76.641	<0.001
	Greenhouse-Geisser	78875.635	2.590	30458.523	76.641	<0.001
Time * Type	Sphericity Assumed	2712.090	16	169.506	1.318	0.199
	Greenhouse-Geisser	2712.090	5.179	523.649	1.318	0.278

Table 7

Tests of Between-Subjects Effects for Juncus *Shoot Counts/Time*

Tests of Between-Subjects Effects					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	3629.962	2	1814.981	.141	.870
Error	180025.578	14	12858.970		

The *Schoenoplectus* plants showed a different result than the *Juncus* plants. The plants in tray ScB had a mean height of 65.54 cm with a standard deviation of 1.64 cm, which was slightly higher than the plants in trays ScC (65.30 1.64) and ScT (62.16 ± 1.64). Like the *Juncus* plants, the result of the Greenhouse-Geisser test showed that TIME was significant and that the TIME*TYPE interaction was non-significant (Table 8). Within-Subjects Contrasts showed that there was a linear trend over time. Again, the Between-Subjects test failed to show an overall significant difference among groups for the TYPE factor (P = 0.306) (Table 9).

Table 8

Significance Levels for Tests of Within-Subjects Effects for Schoenoplectus *Height/Time*

Tests of Within-Subjects Effects for *Schoenoplectus* height/time

Source		Type III Sum of Squares	df	Mean Square	F	Significance
Time	Sphericity Assumed	37816.974	5	7563.395	366.524	<0.001
	Greenhouse-Geisser	37816.974	2.373	15937.891	366.524	<0.001
Time * Type	Sphericity Assumed	371.809	10	37.181	1.802	0.080
	Greenhouse-Geisser	371.809	4.746	78.349	1.802	0.147

Table 9

Tests of Between-Subjects Effects for Schoenoplectus Height/Time

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	212.692	2	106.346	1.311	0.306
Error	973.391	12	81.116		

The *Schoenoplectus* plants in tray ScC had a mean shoot count of 14.061

with a standard deviation of 1.127, which was slightly higher than the plants in trays ScB (12.972 ± 1.127) and ScT (13.322 ± 1.127). Like the *Juncus* plants, the result of the Greenhouse-Geisser test showed that TIME was significant and that the TIME*TYPE interaction appears to be significant showing that the shoot growth was not parallel for the trays over time (Table 10). Within-Subjects Contrasts showed that there was a linear trend over time. The Between-Subjects test failed to show an overall significant difference among groups for the TYPE factor.

Table 10

Results of Tests of Within-Subjects Effects for Schoenoplectus *Shoot Count/Time*

Tests of Within-Subjects Effects of *Schoenoplectus* shoot count/time

Source		Type III Sum of Squares	df	Mean Square	F	Significance
Time	Sphericity Assumed	1070.273	5	214.055	52.132	<0.000
	Greenhouse-Geisser	37816.974	1.816	589.371	52.132	<0.000
Time * Type	Sphericity Assumed	11.744	10	1.174	.226	0.928
	Greenhouse-Geisser	11.744	3.632	3.234	.226	0. 868

Table 11

Results of Tests of Between-Subjects Effects for Schoenoplectus *Shoot Count/Time*

Tests of Between-Subjects Effects					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	18.541	2	9.271	.243	.788
Error	457.315	12	38.110		

The *Spartina* plants in tray SC had a mean height of 48.67 cm with a standard deviation of 3.43 cm, which was higher than the plants in trays ST (42.34 ± 3.13). The Greenhouse-Geisser test showed that TIME was significant and that the TIME*TYPE interaction appeared to be significant (Table 12). This apparent significance was likely due to the loss of one plant in the SC tray due to mortality. Within-Subjects Contrasts showed that that there was a linear trend over time. The Between-Subjects test failed to show an overall significant difference among groups for the TYPE factor (P = 0. 206) (Table 13).

Table 12

Tests of Within-Subjects Effects for Spartina *Height/Time*

Tests of Within-Subjects Effects for *Spartina* height/time						
Source		Type III Sum of Squares	Df	Mean Square	Type III Sum of Squares	Significance
Time	Sphericity Assumed	9638.971	5	1927.794	16.277	<0.001
	Greenhouse-Geisser	9638.971	1.771	5443.485	16.277	<0.001
Time * Type	Sphericity Assumed	127.944	5	25.589	0.216	0.954
	Greenhouse-Geisser	127.944	1.771	72.254	0.216	0.782

Table 13

Tests of Between-Subjects Effects for Spartina *Height/Time*

Tests of Between-Subjects Effects					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	656.007	1	656.007	1.856	0.206
Error	3180.706	9	353.412		

The *Spartina* plants in tray SC had a mean shoot count of 1.82 with a standard deviation of 0.29, which was higher than the plants in the ST group (1.82 ± 0.32). The Greenhouse-Geisser test showed that TIME was significant, but that the TIME*TYPE interaction was also significant, indicating that changes in the number of shoots were not parallel between groups over time (Table 14). Within-Subjects Contrasts showed that there was a linear trend over time. The Between-Subjects test failed to show an overall significant difference among groups for the TYPE factor (P = 0.193) (Table 15).

Table 14

Tests of within-subjects effects for Spartina *shoot counts/time*

Tests of Within-Subjects Effects for *Spartina* shoot counts/time

Source		Type III Sum of Squares	df	Mean Square	F	Significance
Time	Sphericity Assumed	77.454	5	15.491	32.862	<0.001
	Greenhouse-Geisser	77.454	1.252	61.877	32.862	<0.001
Time * Type	Sphericity Assumed	19.666	5	3.933	8.344	<0.001
	Greenhouse-Geisser	19.666	1.252	15.711	8.344	0.011

Table 15

Tests of Between-Subjects Effects for Spartina *Shoot Counts/Time*

Tests of Between-Subjects Effects					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	5.927	1	5.927	1.980	.193
Error	26.941	9	2.993		

The *Spartina* plants in tray SC had a mean leaf count of 7.54 with a standard deviation of 0.31, which was higher than the plants in trays ST (7.12 ± 0.29). The Greenhouse-Geisser test showed that TIME was significant and that the TIME*TYPE interaction was non-significant, although the interaction was significant if the sphericity assumption was assumed (Tables 16). Within-Subjects Contrasts showed that there was a linear trend over time. The Between-Subjects test failed to show an overall significant difference among groups for the TYPE factor (P = 0.343) (Table 17).

Table 16

Significance Levels for Tests of Within-Subjects Effects for Spartina *Leaf Counts/Time*

Tests of Within-Subjects Effects for *Spartina* leaf count/time

Source		Type III Sum of Squares	df	Mean Square	F	Significance
Time	Sphericity Assumed	49.858	5	9.972	6.235	.000
	Greenhouse-Geisser	49.858	1.572	31.724	6.235	.016
Time * Type	Sphericity Assumed	22.653	5	4.531	2.833	.026
	Greenhouse-Geisser	22.653	1.572	14.414	2.833	.101

Table 17

Tests of Between-Subjects Effects for Spartina *Leaf Counts/Time*

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	2.943	1	2.943	1.003	.343
Error	26.401	9	2.933		

CHAPTER IV

DISCUSSION

Wild-Plant Collections

The staining results of wild collected plants indicate that some MS Gulf Coast salt marsh plants are naturally colonized by AMF. Collections of fall/spring marsh plants throughout five consecutive seasons suggest there is a connection between the season and colonization frequency of the plants roots by AMF, with higher colonization rates of collection sites in the spring. Accordingly, the null hypothesis of seasonal independence was rejected and alternate hypothesis was accepted.

The colonization results are interesting because previous studies on *P. australis* showed higher AMF colonization in fall, during late September (Dolinar & Gaberscik 2009). The observed difference in peak seasonal colonization between studies may be due to temperature or the production of key plant hormones which trigger AMF colonization, such as stigolactone.

Another interesting result of the wild-plant collections was that *S. alternifora* was found to be naturally colonized by AMF in the salt marshes of MS. In previous studies in other areas (Daleo et al 2008, McHugh & Dighton, 2004) *S. alterniflora* was thought to be non-mycorhizal. These previous observations may be due to regional differences in AMF colonization or temporal difference in observations (since AMF colonization was found to be seasonal).

It has been suggested that the zonation of the plants in salt marshes of the Gulf Coast may affect the colonization of the plants by AMF. The reason for

this suggestion seems to be the affects that salinity, tidal inundation, and anoxic sediment might have on the AMF's ability to colonize seaward plants. The collections for this study did not support that idea, since *Spartina* the most seaward plant examined was colonized by AMF. There did not appear to be greater colonization of the more landward *Juncus* plants.

Spore-Traps

The results of the *S. alterniflora* spore-traps were very encouraging. Spore-traps have been used in agriculture to promote the colonization of naturally occurring AMF in plants of the same species, but had not previously been used for salt marsh plants. The plants previously uncolonized with AMF in the tray showed a 67% colonization rate, while 89% of the plants which were colonized initially maintained their colonization status over the eight month period. This higher colonization rate (in comparison to the commercial inoculant experiment) suggests that naturally occurring AMF may be better able or more likely to colonize *S. alterniflora* than a commercial inoculant. This result is extremely interesting since *S. alterniflora* was previously thought to be non-mycorhizal in studies by Daleo et al. (2008), and McHugh and Dighton (2004).

All the plants represented in the spore-trap trays were collected from the wild, while the greenhouse experiment involved a mix of wild-collected and nursery-raised plants. Wild plants may have more of an affinity than nursery-raised plants for colonization by AMF. Such enhanced affinity might be related to previous exposure to the same species of AMF that were present in spore-trap trays. High colonization rates within spore-trap trays should encourage further

research into the molecular identification of the AMF species in trays versus those in wild-collected plants. Molecular identification may help isolate the most viable species of AMF for promoting the growth of restoration salt marsh plants under nursery conditions.

The use of spore-trap trays in a nursery setting may prove beneficial for salt marsh restoration projects. Seedlings raised in a nursery could be placed into trays with plants of the same species collected from a healthy salt marsh in order to promote colonization of the nursery seedlings with natural-occurring AMF. Another possible way to encourage natural AMF colonization would be the use of sediment taken from a healthy salt marsh site as a medium in which to grow restoration plants. This sediment would likely contain the AMF spores, as well as other beneficial microbes. The spores in the sediment could be collected through sieving to create an inoculant of salt marsh AMF.

Greenhouse Experiment

The results of the weekly shoot count and height measurements showed that directionality of differences among groups was inconsistent for mean height, shoot, and leaf counts; values were sometimes higher in for the treatment group, sometimes for the base group, and sometimes for the control group. The results of the dry-weight biomass analysis also failed to show any significant differences among any of the groups. The lack of consistent differences in average weekly growth rates were surprising, because it was expected that the treatment group would exhibit increased growth (greater height, more shoots/leaves). Although the treatment group did show slightly higher growth for some variable/plant

combinations, it was lower than the control groups for other combinations. The *Spartina* control group, tray SC, had higher shoot count, above, and below ground dry weight biomass at the end of the experiment. The *Schoenoplectus* control group, tray ScC, had a slightly higher shoot count at the end of the experiment.

There were several problems that arose during the duration of the greenhouse inoculant experiments. There was no SB or Spartina base group for two reasons; one was the lack of un-colonized plants at beginning of experiment. The other reason was because it was previously thought to be non-mycorrhizal so the experiment was only designed to test whether a new kind of AMF inoculants would result in AMF colonization. The greenhouse being unheated may have caused low growth in the winter. Initially the entire experiment was planned to take place in a walk-in incubation chamber but did not due to the unavailability of space in the chamber. Because of the location of the greenhouse there may have been slight differences in light or shading but the trays were set up at the beginning of the experiment to give the most even lighting possible. This too would have been avoided if the incubation chamber was available. It may have been beneficial to have started the experiment in the spring because that is when the plant would grow the most but due the unknown time needed for AFM colonization it was necessary to begin the experiments in the fall. The number of plants in the experiments was much lower than wanted. This was due the lack of un-colonized plants available. In order to solve all the problems with the experiment many un-colonized plants would be needed as well as an

incubation chamber to control environmental factors.

The staining procedure showed that all three species of salt marsh plants in the treatment groups showed some degree of colonization by AMF by the commercial inoculant. The low colonization success for all plant species in the greenhouse experiment was unexpected given the observed natural colonization of wild plants and the observed high colonization rate within spore-trap trays. Observed colonization rates of plants were 33% for JT, 20% for ScT, and 33% for ST. As expected, ScC, ScB, SC, and SB groups showed a complete lack of AMF colonization at the end of the experiment. Lower than expected colonization rates may also explain the lack of significant differences in growth rates and dry-weight biomass.

There are several possible reasons for the low colonization rates of JT, ScT, and ST groups. The innoculant contained *G. intraradices, G. mosseae, G. aggregatum,* and *G. etunicatum*. Although the genus *Glomus* has been shown to be a rapid colonizer and also occurs within the salt marsh environment, the inoculants species are commonly associated with terrestrial agricultural plants. The AMF found in salt marshes of Europe is *Glomus geosporum* (Landwehr et al., 2002). and none of the species in the inoculant have been found there. Thus, salt marsh plants may not be as suitable as hosts to these AMF species, which are common in agriculture settings. AMF are known to not have narrow host specificity, however they can be specific to certain plant groups (Scheublin et al., 2004, Turkman et al., 2008). Another factor that may have influenced the AMF colonization success was that some roots of experimental plants were removed

and examined in December. Also, wild-collected plants showed seasonally low colonization rates in fall, thus colonization success may have also been lower in winter. The addition of additional nutrients may have limited the colonization in the experiment plants as shown in the pervious experiment by Pratt-Zossoungbo (2008) but if this were the case it seems likely that the colonization of the plants in the spore-trap trays would have been affected since they were housed in the same greenhouse and exposed to the same additions. AFM are affected by seasonal changes as well as plant growth cycles. The seasonal temperature changes in the greenhouse may have also affected the colonization of experiment plants but this too would have affected the spore-trap tray colonization.

Conclusion

The results of the wild plant collections showed that *J. roemerianus* and *S. alterniflora* are naturally colonized by AMF on the Gulf Coast and that the frequency of colonization appears to be seasonally influenced. In the greenhouse experiment, all three species of plants in the treatment groups showed at least some degree of colonization, between 20 and 33%. Due to non-independence issues among the groups, the greenhouse experiments can only be considered a pilot study. However, in light of the lack of much difference in growth among the groups in the present study, a fully replicated experiment might not be advisable. Although plants in the greenhouse experiments showed low levels of AMF colonization in the JT, ScT, and ST trays, the spore-trap trays demonstrated that spreading of the natural AMF colonization is possible. The use of AMF spores

isolated spore-trap trays and native AMF spores as planting medium may be beneficial to nursery-raised salt marsh plants for restoration purposes. The uses of molecular techniques to accurately identify the species of AMF found in native salt marsh plants would help to create an inoculant specifically formulated for salt marsh restoration plants.

APPENDIX

TABLES

Table A1

AMF Colonization for Spartina *Plants in Spore-Trap 1*

Plant examined	Results when examined in January 2011
Colonized *Spartina* 1	Positive for AMF
Colonized *Spartina* 2	Positive for AMF
Colonized *Spartina* 3	Positive for AMF
Un-colonized *Spartina* 1	Positive for AMF
Un-colonized *Spartina* 2	Positive for AMF
Un-colonized *Spartina* 3	Positive for AMF

Table A2

AMF Colonization for Spartina *Plants in Spore-Trap 2*

Plant examined	Results when examined in January 2011
Colonized *Spartina* 1	Positive for AMF
Colonized *Spartina* 2	Positive for AMF
Colonized *Spartina* 3	Positive for AMF
Un-colonized *Spartina* 1	Negative for AMF
Un-colonized *Spartina* 2	Positive for AMF
Un-colonized *Spartina* 3	Negative for AMF

Table A3

AMF Colonization for Spartina *Plants in Spore-Trap 3*

Plant examined	Results when examined in January 2011
Colonized *Spartina* 1	Negative for AMF
Colonized *Spartina* 2	Positive for AMF
Colonized *Spartina* 3	Positive for AMF
Un-colonized *Spartina* 1	Positive for AMF
Un-colonized *Spartina* 2	Positive for AMF
Un-colonized *Spartina* 3	Negative for AMF

Table A4

Juncus *Plants in Tray JC Total, Above Ground and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Juncus control 1	16.805	6.528	10.277
Juncus control 2	19.127	7.387	11.740
Juncus control 3	12.390	5.413	6.977
Juncus control 4	17.226	8.141	9.085
Juncus control 5	21.770	9.466	12.304
Means for plants in JC	17.464	7.387	10.077

Table A5

Juncus *Plants in Tray JC, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Juncus control 1	38.8	61.2
Juncus control 2	38.6	61.4
Juncus control 3	43.6	56.4
Juncus control 4	48.3	52.7
Juncus control 5	43.5	56.5
Mean ratios for plants in (JC)	42.3	57.7

Table A6

Juncus *Plants in Tray JB Total, Above Ground and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Juncus base 1	18.312	8.573	9.739
Juncus base 2	14.490	7.657	6.833
Juncus base 3	11.538	4.953	6.585
Juncus base 4	18.285	8.991	9.294
Juncus base 5	11.340	5.166	6.174
Juncus base 6	10.755	4.491	6.264
Means for plants in JB	14.1	6.639	7.482

Table A7

Juncus *Plants in Tray JB, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Juncus base 1	46.8	53.2
Juncus base 2	52.8	47.2
Juncus base 3	42.9	57.1
Juncus base 4	49.2	50.8
Juncus base 5	45.6	54.4
Juncus base 6	41.8	58.2
Mean ratios for plants in (JB)	47	53

Table A8

Juncus *Plants in Tray JT Total, Above Ground and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Juncus treatment 1	14.774	5.938	8.836
Juncus treatment 2	14.564	6.164	8.400
Juncus treatment 3	14.274	5.938	8.336
Juncus treatment 4	12.199	4.364	7.835
Juncus treatment 5	11.814	6.913	4.901
Juncus treatment 6	14.544	3.852	10.692
Mean for plants in JT	13.695	5.528	8.167

Table A9

Juncus *Plants in Tray JT, Above Ground/Total and Below Ground/Total Dry-Weight Measurement*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Juncus treatment 1	40.2	59.8
Juncus treatment 2	42.3	57.7
Juncus treatment 3	41.6	58.4
Juncus treatment 4	35.8	64.2
Juncus treatment 5	58.5	41.5
Juncus treatment 6	26.5	73.5
Mean ratio for plants in (JT)	40.4	59.6

Table A10

Schoenoplectus *Plants in Tray ScC Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Schoenoplectus control 1	20.655	4.223	16.432
Schoenoplectus control 2	18.165	4.233	13.932
Schoenoplectus control 3	19.251	5.706	13.545
Schoenoplectus control 4	16.878	4.149	12.729
Schoenoplectus control 5	26.684	5.955	20.729
Mean for plants in (ScC)	20.327	4.853	15.474

Table A11

Schoenoplectus *Plants in Tray ScC, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Schoenoplectus control 1	20.5	79.5
Schoenoplectus control 2	23.3	76.7
Schoenoplectus control 3	29.6	70.4
Schoenoplectus control 4	24.6	75.4
Schoenoplectus control 5	22.3	77.7
Mean ratio for plants in (ScC)	23.9	76.1

Table A12

Schoenoplectus *Plants in Tray ScB Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Schoenoplectus base 1	18.521	4.077	14.444
Schoenoplectus base 2	17.912	4.973	12.939
Schoenoplectus base 3	21.553	5.192	16.361
Schoenoplectus base 4	19.363	5.077	14.286
Schoenoplectus base 5	15.301	4.294	11.007
Mean for plants in (ScB)	18.530	4.723	13.807

Table A13

Schoenoplectus *Plants in Tray ScB, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Schoenoplectus base 1	22	78
Schoenoplectus base 2	27.8	72.2
Schoenoplectus base 3	24.1	75.9
Schoenoplectus base 4	26.2	73.8
Schoenoplectus base 5	28.1	71.9
Mean ratio for plants in (ScB)	25.5	74.5

Table A14

Schoenoplectus *Plants in Tray ScT total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Schoenoplectus treatment 1	23.498	5.225	18.273
Schoenoplectus treatment 2	20.321	4.450	15.871
Schoenoplectus treatment 3	19.053	4.022	15.031
Schoenoplectus treatment 4	15.671	3.609	12.062
Schoenoplectus treatment 5	16.818	3.816	13.002
Mean for plants in (ScT)	19.072	4.224	14.848

Table A15

Schoenoplectus *Plants in Tray ScT, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Schoenoplectus treatment 1	22.2	77.8
Schoenoplectus treatment 2	21.9	78.1
Schoenoplectus treatment 3	21.1	78.9
Schoenoplectus treatment 4	23	77
Schoenoplectus treatment 5	22.7	77.3
Mean ratio for plant in (ScT)	22.1	77.9

Table A16

Spartina *Plants in Tray SC Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total dry-weight	Above ground dry-weight	Below ground dry-weight
Spartina control 1	Died before measurements		
Spartina control 2	15.628	6.463	9.165
Spartina control 3	15.097	6.436	8.661
Spartina control 4	5.685	1.805	3.880
Spartina control 5	10.300	4.630	5.670
Spartina control 6	7.410	2.903	4.507
Mean for plants in (SC)	10.824	4.447	6.377

Table A17

Spartina *Plants in Tray SC, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Spartina control 1	Died before measurements	
Spartina control 2	41.4	58.6
Spartina control 3	42.6	57.4
Spartina control 4	31.8	68.2
Spartina control 5	45.0	55.0
Spartina control 6	39.2	60.8
Mean ratio for plants in (SC)	41.1	58.9

Table A18

Spartina *Plants in Tray ST Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total dry-weight	Above ground dry-weight	Below ground dry-weight
Spartina treatment 1	10.199	4.910	5.289
Spartina treatment 2	9.170	3.484	5.686
Spartina treatment 3	7.306	3.401	3.905
Spartina treatment 4	10.753	3.868	6.885
Spartina treatment 5	6.381	2.444	3.937
Spartina treatment 6	8.939	3.499	5.440
Mean for plants in (ST)	8.791	3.601	5.190

Table A19

Spartina *Plants in Tray ST, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Aboveground/total dry-weight (%)	Belowground/total dry-weight (%)
Spartina treatment 1	48.1	51.9
Spartina treatment 2	38.0	62.0
Spartina treatment 3	46.6	53.4
Spartina treatment 4	36.0	64.0
Spartina treatment 5	38.3	61.7
Spartina treatment 6	39.1	60.9
Average ratio for plants in (ST)	41.0	59.0

Table A20

Juncus *Greenhouse Experiment AMF Colonization Results,*Only One Root in One Slide Positive for AMF Colonization*

Greenhouse Plant ID	Result (Negative/positive for AMF)
Juncus control 1	Negative
Juncus control 2	Negative
Juncus control 3	Negative
Juncus control 4	Negative
Juncus control 5	Negative
Juncus base 1	Negative
Juncus base 2	Negative
Juncus base 3	Negative
Juncus base 4	Negative
Juncus base 5	Negative
Juncus base 6	Negative
Juncus treatment 1	Positive with very low colonization *
Juncus treatment 2	Negative
Juncus treatment 3	Negative
Juncus treatment 4	Positive with very low colonization*
Juncus treatment 5	Negative
Juncus treatment 6	Negative

Table A21

Schoenoplectus *Greenhouse Experiment AMF Colonization Results*

Greenhouse Plant ID	Result (Negative/positive for AMF)
Schoenoplectus control 1	Negative
Schoenoplectus control 2	Negative
Schoenoplectus control 3	Negative
Schoenoplectus control 4	Negative
Schoenoplectus control 5	Negative
Schoenoplectus base 1	Negative
Schoenoplectus base 2	Negative
Schoenoplectus base 3	Negative
Schoenoplectus base 4	Negative
Schoenoplectus base 5	Negative
Schoenoplectus treatment 1	Negative
Schoenoplectus treatment 2	Positive
Schoenoplectus treatment 3	Negative
Schoenoplectus treatment 4	Negative
Schoenoplectus treatment 5	Negative

Table A22

Spartina *Greenhouse Experiment AMF Colonization Results*

Greenhouse Plant ID	Result (Negative/positive for AMF)
Spartina control 1	Died before staining
Spartina control 2	Negative
Spartina control 3	Negative
Spartina control 4	Negative
Spartina control 5	Negative
Spartina control 6	Negative
Spartina treatment 1	Negative
Spartina treatment 2	Negative
Spartina treatment 3	Positive
Spartina treatment 4	Negative
Spartina treatment 5	Negative
Spartina treatment 6	Positive

Table A23

Schoenoplectus *Plants in Tray ScC Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Schoenoplectus control 1	20.655	4.223	16.432
Schoenoplectus control 2	18.165	4.233	13.932
Schoenoplectus control 3	19.251	5.706	13.545
Schoenoplectus control 4	16.878	4.149	12.729
Schoenoplectus control 5	26.684	5.955	20.729
Mean for plants in (ScC)	20.327	4.853	15.474

Table A24

Schoenoplectus *Plants in Tray ScC, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Schoenoplectus control 1	20.5	79.5
Schoenoplectus control 2	23.3	76.7
Schoenoplectus control 3	29.6	70.4
Schoenoplectus control 4	24.6	75.4
Schoenoplectus control 5	22.3	77.7
Mean ratio for plants in (ScC)	23.9	76.1

Table A25

Schoenoplectus *Plants in Tray ScB Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Schoenoplectus base 1	18.521	4.077	14.444
Schoenoplectus base 2	17.912	4.973	12.939
Schoenoplectus base 3	21.553	5.192	16.361
Schoenoplectus base 4	19.363	5.077	14.286
Schoenoplectus base 5	15.301	4.294	11.007
Mean for plants in (ScB)	18.530	4.723	13.807

Table A26

Schoenoplectus *Plants in Tray ScB, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Schoenoplectus base 1	22	78
Schoenoplectus base 2	27.8	72.2
Schoenoplectus base 3	24.1	75.9
Schoenoplectus base 4	26.2	73.8
Schoenoplectus base 5	28.1	71.9
Mean ratio for plants in (ScB)	25.5	74.5

Table A27

Schoenoplectus *Plants in Tray ScT Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total Dry-weight (grams)	Above ground Dry-weight (grams)	Below ground Dry-weight (grams)
Schoenoplectus treatment 1	23.498	5.225	18.273
Schoenoplectus treatment 2	20.321	4.450	15.871
Schoenoplectus treatment 3	19.053	4.022	15.031
Schoenoplectus treatment 4	15.671	3.609	12.062
Schoenoplectus treatment 5	16.818	3.816	13.002
Mean for plants in (ScT)	19.072	4.224	14.848

Table A28

Schoenoplectus *Plants in Tray ScT, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Schoenoplectus treatment 1	22.2	77.8
Schoenoplectus treatment 2	21.9	78.1
Schoenoplectus treatment 3	21.1	78.9
Schoenoplectus treatment 4	23	77
Schoenoplectus treatment 5	22.7	77.3
Mean ratio for plant in (ScT)	22.1	77.9

Table A29

Spartina *Plants in Tray SC Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total dry-weight	Above ground dry-weight	Below ground dry-weight
Spartina control 1	Died before measurements		
Spartina control 2	15.628	6.463	9.165
Spartina control 3	15.097	6.436	8.661
Spartina control 4	5.685	1.805	3.880
Spartina control 5	10.300	4.630	5.670
Spartina control 6	7.410	2.903	4.507
Mean for plants in (SC)	10.824	4.447	6.377

Table A30

Spartina *Plants in Tray SC, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Above ground/total dry-weight (%)	Below ground/total dry-weight (%)
Spartina control 1	Died before measurements	
Spartina control 2	41.4	58.6
Spartina control 3	42.6	57.4
Spartina control 4	31.8	68.2
Spartina control 5	45.0	55.0
Spartina control 6	39.2	60.8
Mean ratio for plants in (SC)	41.1	58.9

Table A31

Spartina *Plants in Tray ST Total, Above Ground, and Below Ground Dry-Weight Measurements*

Greenhouse Plant ID	Total dry-weight	Above ground dry-weight	Below ground dry-weight
Spartina treatment 1	10.199	4.910	5.289
Spartina treatment 2	9.170	3.484	5.686
Spartina treatment 3	7.306	3.401	3.905
Spartina treatment 4	10.753	3.868	6.885
Spartina treatment 5	6.381	2.444	3.937
Spartina treatment 6	8.939	3.499	5.440
Mean for plants in (ST)	8.791	3.601	5.190

Table A32

Spartina *Plants in Tray ST, Above Ground/Total and Below Ground/Total Dry-Weight Measurements*

Greenhouse Plant ID	Aboveground/total dry-weight (%)	Belowground/total dry-weight (%)
Spartina treatment 1	48.1	51.9
Spartina treatment 2	38.0	62.0
Spartina treatment 3	46.6	53.4
Spartina treatment 4	36.0	64.0
Spartina treatment 5	38.3	61.7
Spartina treatment 6	39.1	60.9
Average ratio for plants in (ST)	41.0	59.0

Table A33

Chi-Square Test of Seasonal Independence of AMF Colonization in Salt Marsh Plants of MS

	Colonized sites	Uncolonized sites	Total
Fall collections	19	11	30
Spring collections	21	0	21
Total	40	11	51
DF=1			
Crit .05, 1=	3.841		
Result of Chi^2	7.770	7.770>3.841, so Ho rejected, Ha accepted	

REFERENCES

Armstrong, W., Wright, E. J., Lythe, S., & Gaynard, T. J. (1985). Plant zonation and the effects of the spring-neap tidal cycle on soil aeration in a Humber salt marsh. *Journal of Ecology, 73*, 323-339.

Bever, J. D., Schultz, P. A., Pringle, A., & Morton, J. B. (2001). Arbuscular mycorrhizal fungi: More diverse than meets the eye and the ecological tale of why. *Bioscience*, *51*, 923-931.

Bird, E. (2008). *Coastal geomorphology, an introduction* (2nd ed.).West Sussex, UK: John Wiley & Sons, Ltd.

Campbell, J., & Biber, P. (2009). *The role of root associated fungi in salt marsh restoration success*. CREST Annual Workshop. Loyola University, New Orleans.

Campbell, N., & Reece, J. (2009). *Biology* (8th ed.). San Francisco, CA: Benjamin Cummings.

Caravaca, F., del Mar Alguacil, M., Torres, P., & Roldan, A. (2005). Microbial activities and arbuscular mycorrhial fungi colonization in the rhizosphere of the salt marsh plant *Inula crithmoides* along a spatial salinity gradient. *Wetlands*, *25*, 350-355.

Craft, C. J., Clough, J., Ehman, S., Joye, R., Park, S., Pennings, H., & Guo, Machmuller, M., (2009). Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. *Frontiers in Ecology and the Environment, 7*, 73–78.

Daleo, P., Alberti, J., Canepuccia, A., Escapa, M., Fanjul, E., Silliman, B. R., Bertness, M. D. & Iribarne, O. (2008). Mycorrhizal fungi determine salt-marsh plant zonation depending on nutrient supply. *Journal of Ecology*, *96*, 431-437.

Dalh, T.E. (1990). *Wetland losses in the United States from 1780's to 1980's*. Washington, DC: United States Department of the Interior, Fish and Wildlife Service.

Dolinar, N.,& Gaberscik, A. (2010). Mycorrhizal colonization and growth of *Phragmites australis* in an intermittent wetland. *Aquatic Botany, 93*(2), 93-98.

Evelin, H., Kapoor, R., & Giri, B. (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Annals of Botany*, *104*, 1263-1280.

Finlay, R. D., & Read, D. J. (1986). The structure and function of the vegetative mycelium of ectomycorrhizal plants. II. The uptake and distribution of phosphorus by mycelial strands interconnecting host plants. *New Phytologist*, *103*, 157-165.

Green, S. B., & Salkind, N. J. (2000). *Using SPSS for Windows and Macintosh: Analyzing and understanding data* (3rd ed.). Upper Saddle River, NJ: Prentice Hall.

Hart, M., & Reader, R. (2002). Taxonomic basis for variation in the colonization strategy of arbuscular mycorrizal fungi. *New Phytologist*, *153*, 335-344.

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

Hildebandt, U., Karlof, M., & Bothe, M. (1999). The zinc violet and its colonization by arbuscular fungi. *Plant Physiology*, *154*, 709-717.

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

Hopkins, C. S., Lugo, A. E., Alber, M., Covich, A., & Van Bloem, S. J. (2008). Forecasting effects of sea-level rise and windstorms on coastal and inland ecosystems. *Frontiers in Ecology and the Environment, 6*, 255–263.

Johnson-Green, P., Kenkel, N., & Booth, T. (2001). Soil salinity and arbuscular mycorrhizal colonization of *Puccinellia nuttalliana*. *Mycological Research*, *105*, 1094-1110.

Keller, E. M., Lajtha, K., & Cristofor, S. (1998). Trace metal concentrations in the sediments and plants of the Danube Delta, Romania. *Wetlands*, *18*, 42-50.

Kiehl, K., Esselink, P., & Bakker, J.P. (1997). Nutrient limitation and plant species composition in temperate salt marshes. *Oecologia, 111*, 325-330.

Landwehr, M., Hildebrandt, U., Wilde, P., Nawrath, K., Toth, T., Biro, B., & Bothe, H. (2002). The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. *Mycorrhiza, 12*, 199-211.

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

Mitsch, W. J., & Gosselink, J. G. (1993). *Wetlands*. (2nd ed.). New York, NY: John Wiley and Sons Inc.

Nybakken, J. (2001). *Marine biology, an ecological approach.* (5th ed.). San Francisco, CA: Benjamin Cummings.

Odum, E., & Barrett, G. (2005). *Fundamentals of ecology* (5th ed.). Belmont, CA: Brooks Coles.

Partyka, M. L., &Peterson, M. S. (2008). Habitat quality and salt-marsh species assemblages along an anthropogenic estuarine landscape. *Journal of Coastal Research*, *24*, 1570-1581.

Pennings, S. C., Callaway, M. R. (1981). Salt marsh zonation: The relative importance of competition and physical factors. *Ecology*, *73*, 681-690.

Pennings, S. C., Grant, M. B., & Bertness, M. D. (2005). Plant zonation in low-latitude salt marshes: Disentangling the roles of flooding, salinity, and competition. *Journal of Ecology, 93*, 159-167.

Pessarakli, M. (2002). *Handbook of plant and crop physiology* (2nd ed.). New York, NY: Marrcel Decker.

Pratt-Zossoungbo, M. (2008). *Mycorrhial fungal associations with salt marsh species Juncus roemerianus*, *Spartina alterniflora*, *Schoenoplectus robustus*, *and Schoenoplectus americanus* (Unpublished master's thesis). The University of Southern Mississippi, Hattiesburg, MS.

Redecker, D. (2008). *Glomeromycota. Arbuscular mycorrhizal fungi and their relative(s). Version 14*. Retrieved from http://tolweb.org/Glomeromycota/28715/2008.01.14

Rooney-Varga, J., Devereux, R., Evans, R., & Hines, M. (1997). Seasonal changes in the relative abundance of uncultivated sulfur-reducing bacteria in salt marsh sediment and in the Rhizosphere of *Spartina alterniflora*. *Applied and Environmental Microbiology*, *63*, 3895-3901.

Rozema, J., Bijwaard, P., Prast, G., & Broekman, R. (1985). Ecophysiological adapatations of coastal halophytes from foredunes and salt marsh. *Vegtatio*, *62*, 499-521.

Scheublin, T.R., Ridgway, K.P., Young, P.W., & van der Heijden M.G.A. (2004). Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Applied Environmental Microbiology*, *70*, 6240-6246.

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

Simenstad, C., Reed, D., & Ford, M. (2006). When is restoration not? Incorporating landscape-scale processes to restore self-sustaining ecosystems in coastal wetland restoration. *Ecological Engineering, 26*, 27–39.

Turkmen, O., Sensoy, S., Demir, S., & Erdinc, C. (2008). Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress. *African Journal of Biotechnology*, *7*, 392-396.

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

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

Umbanhowar, J., & McCann, K. (2005). Simple rules for the coexistence and competitive dominance of plants mediated by mycorhizal fungi. *Ecology Letters*, *8*, 247-252.

Vierheilig, H., Coughlan, A.P., Wyss, U., & Piche, Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology, 64*, 5004–5007.

Zar. (1999). *Biostatistical analysis* (4th ed.). Upper Saddle River, NJ. Prentice-Hall.

Zedler, J. (1984). *Salt marsh restoration: A guidebook for Southern California*. (California Sea Grant Report No. T-CSGCP009). Oakland, CA: California Sea Grant College Program, Institute of Marine Resources, University of California.