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Arbuscular Mycorrhizae Occur in Common *Spartina* Species

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We report the presence of arbuscular mycorrhizae (AM) in *Spartina alterniflora* Loisel roots. Roots were sampled for AM in field-collected and greenhouse-maintained *Spartina patens* (Aiton) Muhl. and *S. alterniflora*, the dominant species of Louisiana's brackish and saline marshes, respectively. Previous reports of AM association in these *Spartina* sp. are limited and conflicting. Field-collected *S. alterniflora* had minimal AM (2.4%), whereas 39.5% of the *S. patens* roots examined were AM colonized. Greenhouse conditions of reduced salinity [3 parts per thousand (ppt)] appeared to increase AM association for *S. patens* compared with field samples. AM occurrence varied significantly among the three sample sites. Our results of low AM association in *S. alterniflora* differ from previous studies and confirm one previous report of AM in *S. patens*. Confirming AM association previously thought to be nonexistent in *S. alterniflora* marshes is a necessary first step in determining if AM influence zonation and competition.

Mycorrhizae are symbiotic fungi of plant roots. Though some fungi are pathogenic (Elmer et al., 2012), mycorrhizae are generally mutualistic; the fungi benefit from plant photosynthate, and the plant has greater access to water and nutrients through the increased root area provided by the fungus. Arbuscular mycorrhizae (AM) form unique structures, such as arbuscules, that penetrate the cell wall of the host plant. These intracellular structures enhance the symbiotic connection of the fungus and host plant.

Symbiosis of AM has been well studied, as Bago et al. (2000) review; however, this symbiosis in wetland species is a relatively new concept. Recently, AM have been recognized as abundant and potentially important for wetland plants in degraded marshes (Kandalepas et al., 2010). AM may influence zonation in salt marshes (Daleo et al., 2008) and increase the ability of plants with AM to compete with non-AM-associated plants (Weishampel and Bedford, 2006). Plants common to salt marshes along the eastern United States form symbioses with AM (Cooke et al., 1993; Hoefnagels et al., 1993), but the presence of AM varies by species (McHugh and Dighton, 2004).

In Louisiana's approximately 4 million acres of coastal marsh, the two dominant plant species of saline and brackish marshes are *Spartina alterniflora* and *Spartina patens*, respectively (Chabreck, 1970; Sasser et al., 2008). National wetland restoration efforts and federal funding have focused on Louisiana wetlands (U.S. Army Corps of Engineers and Louisiana Department of Natural Resources, 2004) due to the high marsh losses there (Barras et al., 2003) and the value of those marshes as an economic resource (e.g.,

fisheries). The AM association with *Spartina* sp., until recently, has been thought nonexistent and therefore not incorporated into ecosystem planning. If AM are associated with these species and influence plant competition and zonation along the coast as previous studies suggest (McHugh and Dighton, 2004; Daleo et al., 2008; Lin et al., 2011), the association may be important to understanding and restoring wetland ecosystems. However, the existence of an AM association within *Spartina* sp. has not been well established. Establishing the presence or absence of an AM association for these species is a first step to determining how AM may influence plant success or failure in wetland ecosystems and their restoration.

Results from studies of AM association with *S. alterniflora* are inconsistent. McHugh and Dighton (2004) reported that commercially added mycorrhizae in *S. alterniflora* increased tillering and plant shoots in that species, whereas Pratt-Zossoungbo and Biber (2009) and Daleo et al. (2008) found no AM with the species. Pratt-Zossoungbo and Biber (2009) were unable to establish AM colonization in *S. alterniflora* roots from coastal Mississippi, although they established colonization in 25% to 37% of *Juncus roemerianus* and *Schoenoplectus americanus* samples. Daleo et al. (2008) reported a lack of AM association in *S. alterniflora* from coastal Argentina. Likewise, Hoefnagels et al. (1993) found no *S. alterniflora* AM association, although it was present in four other species sampled. Hoefnagles et al. (1993) found *S. alterniflora* to be resistant to AM.

Results from studies of AM association with *S. patens* are limited. Only Hoefnagels et al. (1993) reported on AM association in *S. patens*, the species most common in coastal Louisiana
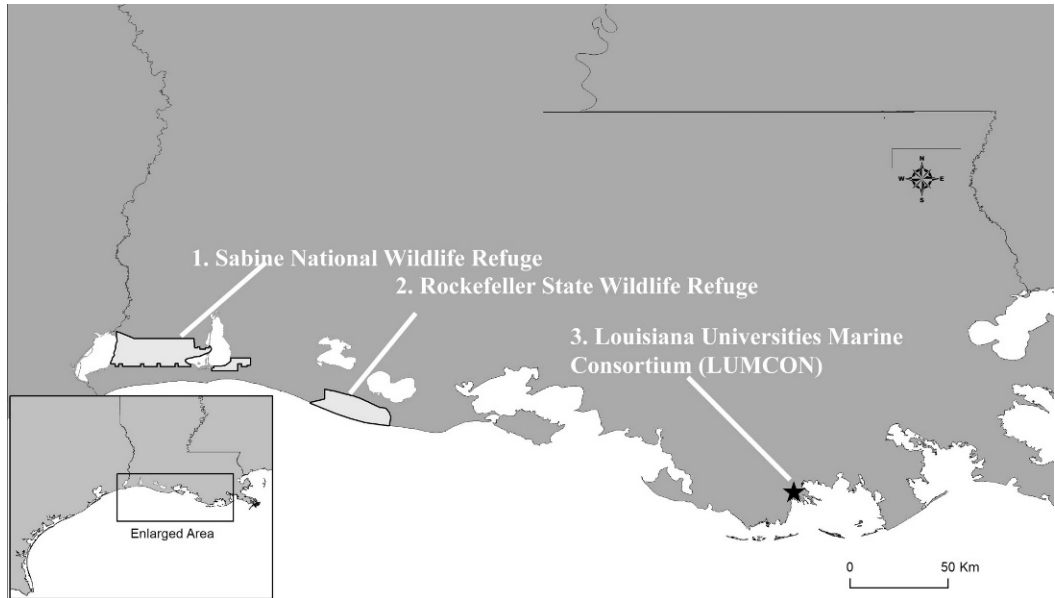
Fig. 1. Three coastal Louisiana marshes where *Spartina* sp. were collected for arbuscular mycorrhizae analysis.

(Chabreck, 1970) and classified as a noxious weed in California and Oregon. The amount of AM in *S. patens* roots varied significantly among locations and with soil properties (Hoefnagles et al., 1993). *Spartina patens* had less AM when grown in soils from *S. alterniflora*-dominated stands than in *S. patens/Distichlis spicata* stand soils (Hoefnagles et al., 1993).

Seasonal and habitat variation may affect mycorrhizae colonization (Cooke et al., 1993; Brown and Bledsoe, 1996; Hildebrandt et al., 2001). Samples of *S. alterniflora* collected in one season could be incorrectly designated as lacking mycorrhizal association, though the symbiosis may be present in unsampled seasons (McHugh and Dighton, 2004). Pratt-Zossoungbo and Biber (2009) suggested that variations in habitat could also be a factor influencing AM associations.

Our primary objective was to determine if AM are associated with the roots of dominant plant species in coastal Louisiana marshes. We included seasonal and habitat (site) variation that could influence AM association. We tested for differences in AM association by species, site, and date. We also tested for species and site differences in plants grown in a greenhouse under reduced-stress conditions.

METHODS

Field.—We collected *S. alterniflora* and *S. patens* within three coastal Louisiana marsh sites (Fig. 1): 1) Sabine National Wildlife Refuge, 2) Rockefeller State Wildlife Refuge, and 3) Louisiana Universities Marine Consortium (LUM-CON). The Sabine refuge southern border is approximately 7 km inland of the Gulf of Mexico shore. Rockefeller and LUMCON are directly along the shore. Sabine is approximately 50 km northwest of Rockefeller, and LUMCON approximately 170 km southeast from Rockefeller. All sites have *S. alterniflora*-dominant saline marsh and *S. patens*-dominant brackish marshes.

At each site, we collected three sediment plugs of each species during four collecting trips: winter 2003, summer 2004, winter 2004, and summer 2005. Winter collections were gathered within a 2-wk period in November, whereas summer collections were conducted during the last week of May or the first week of June of each year. Each plug contained a single plant dug by shovel at least 10 feet from any other sample for a total of 72 samples. We transported plants from each site to the laboratory in plastic bags to prevent cross-contamination. At the laboratory, plants were refrigerated during the week it took to wash soil from the roots and rhizomes, and remove the aboveground biomass.

We separated 10 to 15 roots from each plant plug and stored them in 50% ethanol at 5°C. These were cut into approximately 1-cm sections and placed in scintillation vials with screen tops. The cut roots were first cleared for 20 min in 10% KOH at 90°C, then rinsed three times and stained root pieces were placed on a Petri dish inscribed with grid lines. We evaluated root pieces for

TABLE 1.   Results of a fixed-effects ANOVA of arbuscular mycorrhizal colonization from field-collected samples. Sources of variance are site (Sabine National Wildlife Refuge, Rockefeller State Wildlife Refuge, and Louisiana Universities Marine Consortium), date (winter 2003, summer 2004, winter 2004, and summer 2005), and species (*Spartina patens* and *S. alterniflora*). A probability (*P*) of 0.05 or less was considered statistically significant.

Source	df	Sum of squares	Mean square	F	P
Model $r^2 = 0.94$	20	26,104.8	1,305.2	32.0	<0.0001
Model (residual) error	41	1,672	40.8	-	-
Site	2	604.5	300.7	7.37	0.0018
Date	3	717.4	239.1	5.86	0.0020
Site × date	5	1,061.3	212.3	5.2	0.0009
Species	1	20,251.3	20,251.3	496.6	<0.0001
Site × species	2	733.5	366.7	8.99	0.0006
Date × species	3	553.0	184.3	4.52	0.0079
Site × date × species	4	741.8	185.4	4.55	0.0039

mycorrhizae presence at the location where the root section crossed an inscribed line in the Petri dish. Fifty observations were made for each subsample, and the number of AM found was recorded. The mean AM colonization is an average percentage of AM observed. The experimental unit is the mean percent colonization for a plant; there were three replicate plants for each site–species–date combination. A three-way fixed-effects ANOVA was used to test whether colonization differed among species, site, and date. No samples of *S. alterniflora* were taken from LUM-CON in 2003, or of *S. patens* from Rockefeller in 2004. Type 4 sums of squares, recommended by SAS version 9.1 (SAS Institute Inc., Cary, NC), for data missing a treatment were used in analysis. The model was robust to the normality assumption and data had homogeneity of variance without transformation. To determine which within-effect comparisons were significant, alpha was Bonferroni-method adjusted in least-squares means comparisons to reduce the type 1 error rate (alpha = 0.0009 or 0.05 divided by 58 comparisons). Of the 72 total possible pair-wise comparisons of site, date, and species effects, we had 58 total possible comparisons after omitting missing data and duplicate comparisons (12 date × species, 36 site × species, and 10 date × site comparisons). With this method, both within and among effects were made.

Greenhouse.—We supplemented field-collected samples with plants maintained in a greenhouse to examine whether the controlled environment, which may be less stressful than field conditions, would promote AM colonization. Whole plants were collected from *S. alterniflora* and *S. patens* marshes (same general three sites as for the field collections, Fig. 1, three of each species) in the summer of 2004 by shovel and planted into 18.9-liter pots placed in tubs (378.5-liter Rubbermaid®

Stock Tank). Holes were drilled in the sides of the pots at 2, 5, and 10 cm from the bottom to allow water exchange. Tubs were initially filled to the soil surface with 3 parts per thousand (ppt) salinity water that was mixed from commercial salts (Forty Fathoms®, now Crystal Sea® of Marine Enterprises International, Inc., Baltimore, MD). This salinity is less than brackish or salt conditions (Chabreck, 1970), and intended to reduce plant stress. Water was added weekly to simulate marsh conditions. All plants were fertilized (10:10:4 N:P:K) 2 months after planting. Experimental plants were provided natural indirect light. Temperatures in the greenhouse were kept near ambient using fans to circulate outside air through the greenhouse. We collected three root subsamples from each pot using an approximately 1-cm-diameter microcorer in June 2005, 12 months after collection. We washed soil from the roots and processed them for staining to detect AM using the same process as the field-collected roots. We used a two-way fixed-effects ANOVA to determine whether species or site
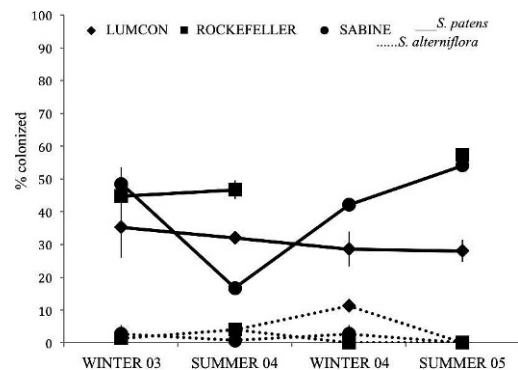

Fig. 2.   Interaction of mean arbuscular mycorrhizal colonization (n = 3) with standard error bars of field-collected species and sites for winter (November) and summer (May–June) over 2 yr.
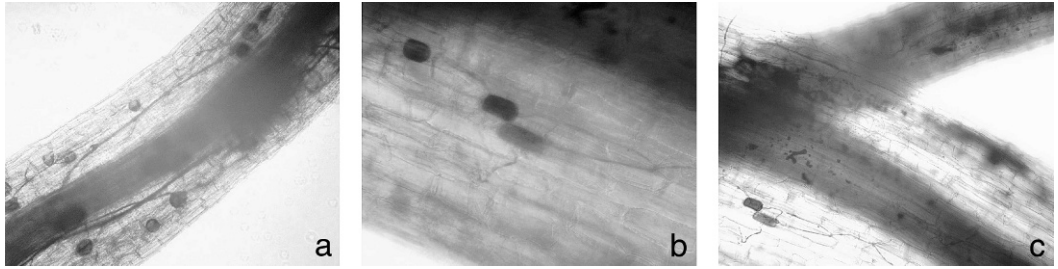
Fig. 3. *Spartina patens* root sections showing stained arbuscular mycorrhizal fungal structures within the root with scale of image increased (a) 100 times; (b) 100 times; and (c) 400 times. Photo: A. Burcham.

differences in the colonization were significant given an alpha of 0.05. For within-effect comparisons, alpha was Bonferonni adjusted for multiple comparisons $(0.05/9 = 0.005)$.

RESULTS

Field.—Mycorrhizal colonization in field samples varied among species, site, and date (Table 1). The mean AM colonization of *S. patens* roots was 39.7%, whereas the mean AM colonization of *S. alterniflora* roots was 2.6%. *Spartina patens* had significantly greater AM colonization than *S. alterniflora* for all sites and dates $(P < 0.0001)$ except Sabine summer of 2004 where AM colonization between the species did not differ significantly (Fig. 2). The most pronounced structures visible were box-shaped or spherical vesicles; hyphae were also visible in some samples (Fig. 3). The roots of *S. patens* from Sabine 2004 had a mean AM colonization of 16.7%, significantly lower than AM colonization from Rockefeller that summer: 46.7% $(P < 0.0001)$. The mean AM colonization of LUMCON roots was 32%, which was not significantly different from the other sites. AM colonization in *S. patens* from Sabine summer 2004 samples was significantly lower than from all other collection dates $(P < 0.0001)$. The drop in colonization is not evident in other marshes from that same time.

Spartina patens winter 2004 Rockefeller samples and *S. alterniflora* LUMCON 2003 were not collected.

Greenhouse.—The colonization of the *Spartina* sp. maintained in the greenhouse for 12 mo was similar to that of field-collected plants; species and site differences in AM colonization were observed $(r^2 = 0.997;$ F = 862; df = 5; $P < 0.0001)$. Evidence of AM in *S. alterniflora* was only observed in collections from Sabine (Table 2). For *S. patens* AM colonization, each site was significantly different $(P < 0.0001)$, with the highest colonization at Rockefeller and the lowest at Sabine (Table 2). The mean percent AM colonization for *S. patens* was significantly higher at all sites than for *S. alterniflora* (Table 3).

DISCUSSION

We observed an association of AM in *S. patens* that has been little reported. Nearly 50% AM colonization was found in *S. patens* roots collected from three sites in coastal Louisiana (39.7%) and growing under greenhouse conditions (48.4%). In contrast, AM association with *S. alterniflora* roots from field and greenhouse was markedly low, but present. Our study differs from previous studies in having found an AM association within *S. alterniflora,* but concur with

TABLE 2. Mean and standard error (STE) of percent arbuscular mycorrhizal (AM) colonization in *Spartina alterniflora* and *S. patens* roots from plants (n = 18) maintained in the greenhouse for 12 mo from Sabine National Wildlife Refuge, Rockefeller State Wildlife Refuge, and Louisiana Universities Marine Consortium (LUMCON), with probability (*P*) of statistically different least-square means of site and species effects and their interaction (site × species).

Site	% AM	STE	Site *P*	S. alterniflora %	STE	S. patens %	STE	Species *P*	Site × species *P*
Sabine	22	9.4		1.3	1.3	43.3	0.7		
Rockefeller	27	12.1		0	0	54	1.15		
LUMCON	24	10.7		0	0	48	1.15		
	24.4	5.9	<0.001	0.4	0.4	48.4	1.6	<0.0001	<0.0001

TABLE 3. Least-squares means comparisons of percent arbuscular mycorrhizal colonization between *Spartina alterniflora* (alt), *S. patens* (patens), and collection sites [Louisiana Universities Marine Consortium (LUMCON), Rockefeller State Wildlife Refuge, and Sabine National Wildlife Refuge]. Mean and standard error (STE) are given; statistically significant differences (*) are <0.003.

	Mean	STE	LUMCON alt	LUMCON patens	Rockefeller alt	Rockefeller patens	Sabine alt	Sabine patens
LUMCON alt	0	0		<0.0001*	1.0	<0.0001*	0.3169	<0.0001*
LUMCON patens	48	1.15			<0.0001*	0.0005*	<0.0001*	0.0033
Rockefeller alt	0	0				<0.0001*	0.3169	<0.0001*
Rockefeller patens	54	1.15					<0.0001*	<0.0001*
Sabine alt	1.3	1.3						<0.0001*
Sabine patens	43.3	0.7						

Hoefnagles et al. (1993) that it may be more resistant to the AM association than other species.

Our sample sizes were sufficient to detect significant differences among site and species, and the magnitude of observed differences was high. Additional studies are needed to determine the function of fungal symbiosis in *S. patens*. If the fungal symbiosis in *S. patens* is beneficial, as it can be in *S. alterniflora* (McHugh and Dighton, 2004), symbiosis could be encouraged through the addition of commercial mycorrhizae in Gulf Coast brackish marshes or discouraged with fungicide application where *S. patens* is a noxious weed, but this is mere speculation.

The difference of AM association we found between species in the greenhouse suggests that the reduced AM in *S. alterniflora* is not a result of the higher salinity typical of the species' habitat. In the application of commercial mycorrhizae, McHugh and Dighton (2004) showed that increasing salinity from 0 ppt to 7 ppt reduced AM in *S. alterniflora*. Conversely, we maintained *S. alterniflora* at 3 ppt, a salinity lower than the 5–18 ppt range of saline marshes in coastal Louisiana (Chabreck, 1970). We did not have a salinity treatment, but did find that reducing salinity for *S. alterniflora* did not increase AM association.

With the exception of the 2004-summer sample, Sabine and Rockefeller sites (both in southwest Louisiana) demonstrated the greatest AM colonization, whereas LUMCON marsh samples (from southeast Louisiana) had consistently lower AM colonization. However, the greenhouse-maintained plants from LUMCON had a 17% greater AM colonization rate than the average of plants from the field at LUMCON. It is possible that colonization was low in the field due to higher salinity or plant competition, both of which would have been reduced under our greenhouse conditions. Although we did not

monitor field conditions, the salinity of 3 ppt maintained in the greenhouse was lower and less variable than what would occur in brackish and saline marshes.

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