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CONSEQUENCES OF MYCORRHIZAL COLONIZATION FOR *PIRIQUETA* MORPHOTYPES UNDER DROUGHT STRESS

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Field and greenhouse studies have shown that arbuscular mycorrhizal fungi (AMF) can improve plant growth in environments with restricted water availability. The benefits of AMF symbiosis vary among plant species, but the extent to which AMF-mediated drought tolerance varies among subspecific taxa remains poorly understood. In this study, we examine differences in AMF response among three recently diverged, ecologically heterogeneous plant taxa (morphotypes) within the *Piriqueta cistoides* ssp. *caroliniana* complex. We performed a greenhouse experiment using cuttings of each morphotype inoculated in field-collected soil to test for inoculum source effects of AMF on plant growth under drought. Correlation between AMF colonization and plant performance under drought was significant for all three morphotypes but was strongest for *viridis* morphotype; this group is associated with mesic, low-phosphorous soils of south Florida slash pine flatwoods. Compared to inocula obtained from other morphotypes' regions, the AMF obtained from one of the most arid habitats (*caroliniana*) promoted an equal or greater amount of growth in host plants despite relatively low levels of root colonization. These findings suggest that both genetic divergence among morphotypes and the source of AMF inoculum affect plant growth under drought in *P. c.* ssp. *caroliniana* complex.

Keywords: arbuscular mycorrhizal fungi (AMF), Florida, hybridization, leaf morphology, *Piriqueta cistoides* ssp. *caroliniana*, Turneraceae.

Introduction

Mycorrhizal fungi occur as symbionts in association with most terrestrial plants and can be essential to successful growth and reproduction (Harley and Smith 1983; Lu and Koide 1994; Smith and Smith 1997; Smith and Read 2008). Mycorrhizal symbioses appear to play important roles in mitigating the effects of various environmental stresses, including pathogens (Newsham et al. 1995), heavy metals (Schutzendubel and Polle 2002; Vivas et al. 2003), soil salinity (Ruiz-Lozano and Azcon 2000; Daei et al. 2009), and drought (Ruiz-Lozano et al. 1995; Augé 2001). Genetic and environmental differences are known to influence host plant responses to arbuscular mycorrhizal fungi (AMF) (Johnson et al. 1997a, 2010; Parke and Kaeppler 2000; Hoeksema et al. 2010), and the study of interactions between different plant and AMF species remains an active area of research (Smith and Read 2008). The evolution of different degrees of reliance on AMF has been identified among plants that grow in soils with contrasting phosphorous (Schultz et al. 2001; Olson and Tyler 2004) and nitrogen contents (Johnson et al. 2010). Differences in drought tolerance have been identified among cultivars of wheat inoculated with the same species of AMF (Al-Karaki 1998), but overall, relatively little is known of the consequences for intraspecific diversification among host plants in relation to their reliance on AMF.

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Functional divergence in the AMF symbiosis has been found among host species and also occurs among different AMF species (Gazey et al. 2004) and ecotypes (Antunes et al. 2011). Previous research has shown that the degree to which AMF can mitigate drought stress varies among fungal strains (Allen et al. 1995), and other studies have shown greater levels of drought tolerance attained by inoculating plants with AMF spores obtained from arid regions (Ruiz-Lozano et al. 1995; Gange and Brown 2001; Marulanda et al. 2003). The study of interactions among plant species and AMF, and the capacity for this interaction to promote drought tolerance in the plants is an area of significant importance in the research fields of plant ecology and agriculture (Augé 2001; van der Heijden et al. 2004).

Recently diverged plant taxa can provide an opportunity to investigate the evolution of AMF-mediated growth under drought. This study utilizes morphotypes in the *Piriqueta cistoides* ssp. *caroliniana* complex (Arbo) (Turneraceae), a group of closely related perennial plants that occur in tropical and subtropical regions of North and South America, and the Caribbean. Two ecologically distinct parental morphotypes from North America have given rise to a naturally occurring hybrid zone spanning central Florida. The *viridis* morphotype (V) occurs along southern Florida in periodically flooded calcareous soils with low phosphate content that supports pine/palmetto vegetation. The *caroliniana* morphotype (C) is found in southern Georgia and northern Florida in sandhill scrub and slash pine habitats. These two parental morphotypes are genetically distinct, as revealed by nuclear and cpDNA

markers (Martin and Cruzan 1999; Maskas and Cruzan 2000). Between the north and south regions exists a broad hybrid zone that extends across central Florida and probably formed within the last 5000 years (Maskas and Cruzan 2000; Cruzan 2005). Populations towards the center of the hybrid zone are genetically and morphologically uniform. These advanced-generation recombinant hybrids (H morphotype) were derived from interbreeding between the C and V morphotypes (Cruzan and Rhode 2004; Rhode and Cruzan 2005; Machado and Cruzan 2010). The recent divergence among morphotypes presents an opportunity to examine changes in the ability of these plants to benefit from associations with AMF strains that are associated with different environments.

Plants in the *P. c. caroliniana* species complex are associated with habitats that vary in the duration and intensity of drought (Martin and Cruzan 1999; Rhode and Cruzan 2005; Picotte et al. 2007, 2009). Morphological and functional traits associated with the hybrid morphotype confer superior drought tolerance (Picotte et al. 2007), but the role of AMF in drought mediation for this and other taxa has not been tested. In a previous study (Sochacki 2010), we found that drought tolerance of plants of the *P. c. ssp. caroliniana* complex improved when inoculated with a commercially available mixture of AMF spores. Here, we assess plant growth under drought among morphotypes and hybrids when inoculated with field soil AMF found in association with the rhizospheres of naturally occurring populations of each morphotype. We test the hypotheses that (1) plants of the different morphotypes will differ in their degree of association and dependence on AMF under drought; (2) the two morphotypes from the more arid regions (C and H) will be more similar in their responses to AMF than the morphotype from the more mesic habitat (V); and, (3) AMF derived from arid regions would be more beneficial to plant growth under drought than AMF from the more mesic habitat.

Material and Methods

Plants and Experimental Design

Seeds represented a minimum of 24 maternal families collected across at least 5 populations from each of the C, V, and H morphotypes. Seeds were germinated in foil-wrapped petri dishes on Whatman no. 1 filter paper discs (10 cm diameter) moistened with 5 mL of distilled water and incubated at ambient greenhouse temperatures for 4 wk. Seedlings were transplanted into peat pellets and allowed to establish under mesic conditions and an ambient temperature of approximately 28°C for an additional 8 wk.

Cuttings were used to provide a robust experimental design by replicating individuals across treatments. We used cuttings because, compared to cultivating new plants from limited field accessions of *Piriqueta* seed, this is a consistent and low-cost method for increasing experimental replication. Cuttings were prepared by dipping stem lengths of one node into Rootone powder (indole-3-butyric acid; 0.1%) to promote root development and then inserting each cutting into a peat pellet. Cuttings were grown for 4 wk with regular watering before being bare-rooted and transplanted into

treatment trays containing horticulturally sterile sand with or without an amendment of field soil as AMF inoculum. Volumetric differences between field-moist and air-dried soils resulted in unequal volumes of field soil amendments applied to the greenhouse treatments. Air-dried field soil amendments consisting of 0.9 L field soil from the *caroliniana* region, 1.0 L field soil from the hybrid zone, or 1.4 L field soil from the *viridis* region were applied to three sets of 2 trays containing 13.5 L of factory-washed, horticulturally sterile, graded quartz sand. Two additional trays of *Piriqueta* plants served as unamended, noninoculated controls and received an additional 1.5 L of sand in lieu of a field soil application. All trays were brought to a final volume of 15.0 L with sand. A total of 224 plants were randomized by position in 8 trays of 28 cuttings each in an incomplete block design, with cuttings of the same morphotype evenly distributed across trays and treatments. Cuttings were spaced approximately 8 cm apart from each other and from the sides of the tray.

Measurements of plant growth and leaf morphology (including plant height, leaf number, leaf length, and leaf width) were made at transplant and again after the plants established under mesic conditions for 4 wk. After establishment, growth and leaf morphology were measured every 2 wk for a period of 12 wk. Shoots and roots for each plant were separated at the end of 12 wk and dried at 60°C for a minimum of 48 h, and dry weights were determined. A histocassette filled with a sample of each plant's root system was collected and stored for up to 4 wk in refrigerated lactoglycerol before being processed into slides for observation of AMF (Brunnett et al. 1996).

Over the course of the experiment, the greenhouse average daytime temperature was 27°C (range 26°–35°C), with a minimum night temperature of 21°C. Plants were grown under a drought stress regimen, maintained by watering in each tray to saturation capacity upon the earliest observation of non-permanent wilting (Allen and Boosalis 1983) in at least 10% (≥ 3) of the plants in a tray. This is a commonly used method of imposing drought stress (Tyree et al. 2003; Ebdon and Kopp 2004; Du et al. 2009). Plants were checked for signs of drought stress daily and watered every 2 to 5 d. All trays were rotated in a clockwise sequence each week to minimize environmental effects related to greenhouse bench position.

Field Soil

Field soil used in the experiment was collected from the contrasting habitats associated with populations of the C and V morphotypes in Florida, as well as from the hybrid zone where populations of H morphotype occurred (table 1). Using a soil corer, a minimum of 1.0 L of soil was collected from the top 10 cm of the soil surface (O and A horizons) at each of the 16 field sites surveyed in July 2007. A portion of the soil collected from each site (200 g dry weight) was submitted for soil chemistry analysis to A and L Laboratories (Memphis, TN) to measure soil pH, exchangeable phosphate (PO_4), potassium (K), calcium (Ca), magnesium (Mg), cation exchange capacity, percent organic matter (OM), and estimated nitrogen released (ENR). A 30-g sample of dry soil

Table 1
Availability of Phosphate (P) and Calcium (Ca), Percent Organic Matter (OM), and Estimated Nitrogen That Will Be Released from Organic Matter (ENR) for Soils from *Piriqueta* Sites Used for Arbuscular Mycorrhizal Fungi Inoculum

Soil type	<i>n</i>	P (PO ₄) (ppm)	Ca (ppm)	OM (%)	ENR
<i>P. caroliniana</i>	5	44.60 ± 25.13 ^A	588.40 ± 168.98 ^{AB}	2.38 ± .14 ^A	91.60 ± 2.71 ^A
Hybrid zone	6	34.67 ± 8.01 ^A	439.00 ± 132.46 ^B	1.37 ± .07 ^B	71.33 ± 1.33 ^B
<i>P. viridis</i>	5	4.20 ± .86 ^B	2746.20 ± 1111.01 ^A	1.56 ± .27 ^B	75.20 ± 5.31 ^B
<i>F</i> value		6.87	5.9	10.35	10.35
<i>P</i> > <i>F</i>		.0092	.0150	.002	.002

Note. Different letters indicate Tukey-Kramer separated means among the morphotypes. Values indicate mean ± 1 SE. ENR is reported as pounds per acre per season.

from each collection site was passed through a series of soil sieves with progressively finer mesh, and a suspension was made of the spores in the 38–250 μm soil fraction, known to contain the majority of AMF spores in natural systems (Brundrett et al. 1996). Spores in each sample were then enumerated microscopically.

AMF Quantification

Root samples were stained for AMF with trypan blue in a modification of previously established methods (Giovannetti and Mosse 1980). Samples in histological tissue cassettes were immersed in a vigorously boiling solution of 10% potassium hydroxide for 15 min under a fume hood. Cassettes were then rinsed with tap water to remove excess base and placed in 2% hydrochloric acid for 90 min at room temperature to acidify chitinous tissues for staining. Once primed for staining, tissue cassettes were placed in a 0.05% (w/v) solution of trypan blue in lactoglycerol (1:1:1 lactic acid:glycerol:water) for 60 min at room temperature. Following staining, roots were transferred to a solution of lactoglycerol solution for destaining and storage for a minimum of 2 h until slides could be made and the extent of AMF colonization scored.

Observations of stained specimens were made at ×100 magnification while scanning for signs of AMF colonization. Magnification was increased to ×400 to improve visualization of arbuscules within root cortical cells. Colonization of roots by AMF was assessed using the magnified intersections method (McGonigle et al. 1990), scoring the number of AMF structures (hyphae, vesicles, and arbuscules) per 100 reticle intersections encountered while scanning across root segments lined in parallel across the length of each slide. The total number of positive hits was tallied and reported as total colonization for each root slide.

Data Analysis

We tested for the effects of morphotype and soil inoculum source on measures of plant growth, harvest biomass, and leaf shape. Differences in plant growth and harvest biomass in response to the field soil AMF treatments were analyzed using PROC MIXED in SAS v9.2 (SAS 2007), specifying tray nested within treatment as a random factor. Plant size (the

natural log of plant height multiplied by the number of nodes present; Picotte et al. 2007) was calculated for subsequent use in the plant morphological analysis of overall plant growth. Leaf morphology was analyzed as leaf area (the product of leaf length and width) and leaf shape (leaf width divided by its length; Martin and Cruzan 1999; Picotte et al. 2009). Leaf size (calculated as the product of length and width) at the time of transplant was recorded for each cutting for use as a covariate with ANOVA testing for plant growth and biomass. Initial leaf shape was used instead of initial size in analyses of covariance for leaf shape. By including initial size measurements as covariates in the model we specifically test for changes in the response variables (plant size, leaf size, or leaf shape) in response to the treatments. All plant morphological, plant biomass, and AMF colonization (total colonization, as well as individual AMF structural components) measures were square root or natural log transformed as needed to produce distributions that were approximately normal (Sokal and Rohlf 1995).

Results

AMF Colonization

Plant morphotype was not a significant predictor of AMF colonization (table 2). Although plants with greater biomass had higher AMF colonization levels, field soil treatment was the most important factor in explaining colonization. Across all morphotypes, the lowest AMF colonization occurred in plants inoculated with field soil from the *caroliniana* region (fig. 1; table 3). Initial cutting size at the start of the experiment was negatively associated with arbuscule counts (table 2). ANCOVA modeling detected a difference in mean vesicle counts between the 2 trays of *caroliniana* field-soil treatment; however, a pooled variance *t*-test found this difference was not significant ($P = 0.2954$). Root hyphal colonization was highly correlated with total AMF colonization ($r = 0.96$), as were arbuscule and vesicle counts ($r = 0.82$ and $r = 0.81$, respectively). Incidental levels of AMF hyphae (0.8%) were detected among plants in the control treatment trays. These levels were significantly lower than in treatments where colonization resulted from field soil inoculation ($df = 195$, $t = -11.01$, $P < 0.0001$), and no arbuscules or vesicles were observed in the uninoculated control treatment. Spore den-

Table 2**Results of ANCOVA for Total Arbuscular Mycorrhizal Fungi (AMF) Colonization and Mycelia Structures ($n = 150$)**

Source	df	Total AMF	Hyphae	Vesicles	Arbuscules
Soil	2	27.19****	38.66****	17.38****	14.98****
Tray (soil)	3	2.18 ^{ns}	2.17 ^{ns}	2.91*	2.27 ^{ns}
Shoot biomass	1	6.03*(+)	4.75*(+)	4.95*(+)	6.73*(+)
Root biomass	1	.12 ^{ns}	.04 ^{ns}	.04 ^{ns}	.78 ^{ns}
Morphotype	2	.84 ^{ns}	.80 ^{ns}	1.54 ^{ns}	.29 ^{ns}
Morph \times soil	4	.73 ^{ns}	1.01 ^{ns}	.75 ^{ns}	.76 ^{ns}
Initial	1	3.34 ^{ns}	2.55 ^{ns}	3.60 ^{ns}	5.82*(-)
Error MS	135	1.34	.89	1.04	1.13
Model P		<.0001	<.0001	.0001	.0004
Model F		4.74	6.43	3.40	3.03

Note. The data shown are in response to source of soil inoculum (table 1), tray within treatment, plant biomass, morphotype, the interaction between morphotype and soil treatment, and initial measurements in *Piriqueta* morphotypes grown under drought conditions. For covariates with significant effects, slope is indicated as positive or negative. Control trays were dropped from this analysis to allow for a more robust comparative analysis of colonization resulting from inoculation with field soils.

* $P \leq 0.05$.

*** $P \leq 0.001$.

**** $P \leq 0.0001$.

sity associated with the *caroliniana* field soil treatment (4.9 spores/cm³) was more than twice that of the hybrid zone (2.3 spores/cm³) or *viridis* region (1.9 spores/cm³) field soil treatments, suggesting that initial differences in spore densities were not directly related to differences in colonization levels observed in the greenhouse.

Field Soil Chemistry

Soils from the *caroliniana* region contained the highest levels of OM and ENR, as well as the highest concentrations of PO₄ (table 1). Soil from the hybrid zone had levels of PO₄ similar to those determined for the *caroliniana* region, had OM and ENR levels comparable to those found in soils from the *viridis* region, and contained less calcium than soils from either other region. Soils obtained from the *viridis* region had the highest calcium levels and lowest phosphorous levels.

Inputs of mineral nutrients associated with each field soil treatment were calculated (v/v) based on field soil chemistry of inoculum from each region. Among the soil treatments, soil chemistry values were calculated to be 2.7 ppm PO₄, 1.3 ppm K, 34.4 ppm Ca, 3.8 ppm Mg, and 0.14% OM for soil from the *caroliniana* region; 0.3 ppm PO₄, 0.7 ppm K, 165.4 ppm Ca, 3.24 ppm Mg, and 0.11% OM for soil from the *viridis* region; and 3.1 ppm PO₄, 1.4 ppm K, 39.0 ppm Ca, 3.1 ppm Mg, and 0.13% OM for soil from the hybrid zone.

Plant Growth Responses

Compared to the control plants, shoot dry mass increased 76.2% among plants receiving a field soil treatment ($N = 195$, $t = -4.62$, $P < 0.0001$). Among all plants, shoot dry mass was greatest among the hybrids ($df = 2$, 186 , $F = 53.33$,

$P < 0.0001$; fig. 2); however, biomass among the hybrids was not significantly greater than among plants of the C morphotype (contrast: $df = 1$, 187 , $P = 0.1182$). Shoot dry mass at harvest was positively correlated with plant size ($r = 0.72$, $P < 0.0001$), as well as with the total numbers of buds ($r = 0.49$, $P < 0.0001$) and flowers ($r = 0.45$, $P < 0.0001$) produced by each plant over the course of the experiment. Root dry mass generally increased with the addition of field soil and was strongly correlated with shoot dry mass ($r = 0.81$, $P < 0.0001$). Repeated-measures analyses of plant size and leaf shape found these traits were significantly associated with the morphotype, field soil treatment, and initial measurements (table 4). Leaf area over time was associated only with plant morphotype and initial area.

Shoot dry mass increased with the addition of field soil across morphotypes (fig. 2). The greatest overall increase in biomass in response to inoculation with field soil occurred for plants of the V morphotype inoculated with soil from the *caroliniana* region (+144% vs. control). Shoot dry mass for the C morphotype also increased the most when plants were grown with field soil from the *caroliniana* region (+77% vs. control). Biomass among plants of the H type increased most in response to inoculation with field soil from the *viridis* region (+106% vs. control). Although there were differences in shoot dry mass between plants cultivated with and without field soil amendments (fig. 2), differences in biomass among soil treatments within each morphotype were not statistically significant. For shoot dry mass at harvest, slopes for the relationship with total AMF colonization differed among morphotypes ($df = 3$, 196 , $F = 16.47$, $P < 0.0001$), with the V morphotype having the strongest response (slope = 0.14 ± 0.043 , $P = 0.0015$, $N = 66$), followed

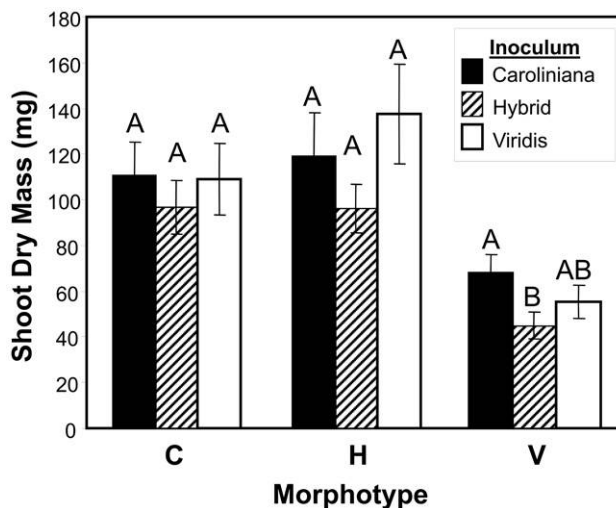


Fig. 1 Total arbuscular mycorrhizal fungi colonization for morphotypes of the *Piriqueta cistoides* ssp. *caroliniana* complex inoculated with field soil from sites associated with the *caroliniana* (C), *viridis* (V), and hybrid zone (H) regions. Different letters represent Tukey-Kramer separated means within each morphotype as determined from ANOVA testing of transformed data. Error bars indicate 1 SE.

Table 3

Distribution of Percent Root Colonization (Total Arbuscular Mycorrhizal Fungi [AMF] and by Structure) at Harvest and Results of One-Way ANOVA for Differences in Colonization after Inoculation with Soil from Different Sources

Innoculum	<i>n</i>	AMF	Hyphae	Vesicles	Arbuscules
<i>Piriqueta caroliniana</i>	53	12.2 ± 2.0 ^C	5.5 ± .9 ^B	3.0 ± .6 ^B	4.4 ± 1.0 ^B
Hybrid	54	43.3 ± 3.8 ^A	22.6 ± 2.1 ^A	10.4 ± 1.3 ^A	10.8 ± 1.3 ^A
<i>Piriqueta viridis</i>	47	31.9 ± 3.3 ^B	17.5 ± 1.7 ^A	7.9 ± 1.2 ^A	8.2 ± 1.2 ^A
Model <i>F</i>		29.28	29.28	5.9	10.35
<i>P</i> > <i>F</i>		<.0001	<.0001	.015	.002

Note. Different letters indicate grouping of means based on Tukey-Kramer multiple range tests. Control trays were dropped from this analysis to allow for a more robust comparative analysis of colonization resulting from inoculation with field soil. Values indicate mean ± 1 SE.

by the H (slope = 0.13 ± 0.049, *P* = 0.0107, *N* = 71) and C (slope = 0.09 ± 0.038, *P* = 0.0162, *N* = 60) morphotypes.

Plants of the V morphotype receiving a field soil treatment had greater leaf areas and narrower leaf shapes at harvest (i.e., leaves were longer); both measures were positively correlated with shoot dry mass in this morphotype (area: *r* = 0.39, *P* = 0.0032; shape: *r* = 0.29, *P* = 0.0324). Increased leaf area and longer leaves in response to different field soil amendments were not observed in either the C or H morphotypes (*P* > 0.05 for all). Among plants of the V morphotype, root/shoot (R/S) ratios decreased with total AMF colonization (df = 3, 46, *F* = 3.52, *P* = 0.0223). Subsequent ANCOVA modeling of the different AMF structures found that arbuscule frequencies had the strongest predictive power for R/S ratios in the V morphotype (df = 3, 46, *F* = 5.00, *P* = 0.0044). The reduced R/S ratios were also accompanied by increases in shoot dry mass corresponding to total AMF colonization, indicating the reduction in R/S ratios was a consequence of increased aboveground rather than reduced belowground biomass. Associations between AMF colonization (or any of the different AMF structures) and R/S ratio or shoot dry mass were not significant in any of our analyses for the C and H morphotypes (*P* > 0.05 for all).

For plants of the H morphotype, a statistically significant interaction occurred between AMF colonization and root dry mass at harvest for the different soil inocula (df = 2, 42, *F* = 3.64, *P* = 0.0349). The greatest root dry mass among the hybrids occurred in combination with soil from the *viridis* region, though the relationship between AMF colonization and root dry mass (adjusted for initial plant size) was not significant in this case (df = 1, 16, *F* = 2.40, *P* = 0.14). There were no strong interaction effects on other plant traits based on the source of the soil inoculum.

Discussion

We found consistent increases in growth under drought stress across all plant morphotypes when inoculated with field soil containing AMF spores; however, in some cases growth improvement depended on the source of field soil inoculum. The plants from the two more arid habitats (C and H) attained greater biomass under drought conditions compared to the V morphotype, but their responses to AMF colonization were more heterogeneous. Colonization of roots by

the fungi conferred the greatest drought tolerance benefit to plants of the V and H morphotypes, while the effects on biomass of C morphotype plants with increased colonization was much lower. This finding supports the hypothesis that genetically determined differences among closely related plant taxa play a key role in mediating plant-fungal symbiotic interactions (Parniske 2004; Balestrini 2006; Smith and Read 2008), and highlights the potential for regional differences in soil AMF composition to have significant effects on plant growth (Gazey et al. 2004; Seifert et al. 2009; Johnson et al. 2010). Our study suggests that intraspecific genetic differences among plants can significantly affect the extent to which AMF symbiosis is capable of mitigating drought stress in host plants. Colonization of plants by different ecotypes of AMF may also have a significant impact on plant growth under drought. These results are, to our knowledge, the first to document

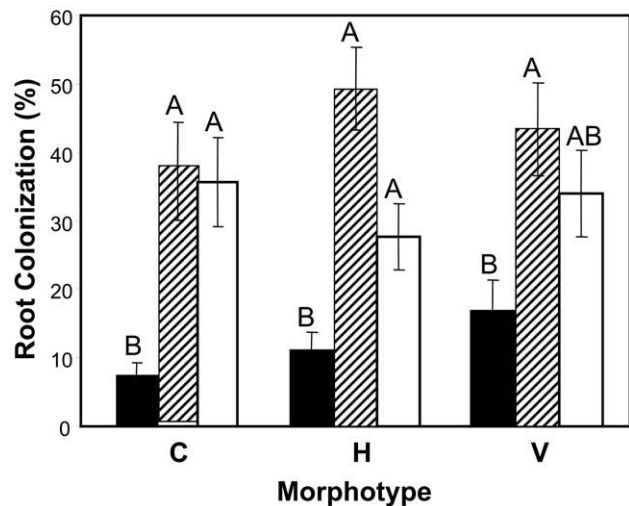


Fig. 2 Shoot dry mass for morphotypes of the *Piriqueta cistoides* ssp. *caroliniana* complex inoculated with field soil from sites where the *caroliniana* (C), *viridis* (V), and hybrid zone (H) morphotypes occur. Different letters represent different means (separated using the Tukey-Kramer method) within each morphotype as determined from ANOVA testing of transformed data. Error bars indicate 1 SE. Treatments are the same as for fig. 1.

Table 4

Repeated-Measures ANOVA Results (<i>F</i> Values)				
Source	df	Plant size	Leaf area	Leaf shape
Morphotype	2	24.64****	204.23****	10.43****
Innoculum	3	16.18****	2.38 ^{ns}	3.09*
Tray (treatment)	4	1.39 ^{ns}	1.15 ^{ns}	1.65 ^{ns}
Morph × treatment	6	.73 ^{ns}	.54 ^{ns}	.48 ^{ns}
Initial	1	114.19****(+)	53.76****(+)	267.44****(+)
Error MS	161	987.22	1.09	.01
Model <i>P</i>		<.0001	<.0001	.0006

Note. The data are for effects of plant morphotype, soil inoculum (including control treatment), the interaction of plant morphotype and soil treatment, and initial measurements on plant size, leaf area, and leaf shape in *Piriqueta* growing under drought conditions. Results are reported only for plants receiving field soil. Plus and minus signs indicate direction of covariate slopes.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

**** $P \leq 0.0001$.

intraspecific differences in AMF-mediated drought tolerance occurring among recently diverged lineages.

A caveat in the interpretation of this experiment is the potential for confounding effects of soil chemistry on plant growth arising from the application of field soil along with naturally occurring AMF. Plant growth may benefit from the application of organic matter, capable of slowly releasing nutrients for an extended period of time (Goyal et al. 1999; Essington 2003). It is possible that the enhanced growth of V morphotype plants in *caroliniana* region field soil could be attributed to greater level of nutrients (particularly exchangeable PO_4) added incidentally as part of the field soil treatment. If growth responses were due to added nutrients then we would expect a similar response after inoculation with soil from the H region, which had similar nutrient levels, but this was not the case. Overall, the growth responses of plants in these treatments are not associated with nutrient additions. On the other hand, regression analyses for the morphotypes support a direct relationship between AMF colonization and shoot dry mass, appearing to be strongest among plants of the V morphotype. It is possible that the general increase in growth across morphotypes in response to soil inoculation is partly due to nutrient addition, but differences we found in plant growth among morphotypes under drought were more likely the consequence of AMF colonization.

A higher level of exchangeable PO_4 was introduced with field soil from the *caroliniana* region, which could explain why AMF colonization was significantly lower in this treatment (Menge et al. 1978; Schwab et al. 1983). However, AMF colonization resulting from the hybrid zone field soil was nearly four times greater, despite a comparable level of exchangeable PO_4 in that treatment. Plants grown with field soil from the hybrid zone also had greater levels of colonization than those grown with phosphate-deficient soil from the *viridis* region. These differences in colonization appear to reflect differences in colonization propensity by AMF from different regions, but these soils also differ in their chemistry. While it is widely accepted that the soil chemical environment can strongly influence the ability of AMF to success-

fully colonize host plants (St. Clair 2005; Smith and Read 2008), our understanding of how the underlying mechanisms is limited at best and merits further study.

Plants of all three morphotypes demonstrated increased vegetative growth when inoculated with field soil AMF, indicating an enhanced capacity for tolerating drought (Gurevitch et al. 2002); however, the mechanism underlying this response remains ambiguous. It is possible that the increased biomass we observed was associated with greater soil hyphal density; soil-colonizing hyphae radiating from AMF-colonized roots often play an important role in the stabilization and promotion of soil-stable aggregates and increasing the water retentive properties of soil (Miller and Jastrow 1990). While extraradical hyphal density was found to be strongly correlated with frequencies of intraradical hyphae in *Phaseolus vulgaris* (Jastrow 1998; Augé et al. 2003), this trend was not observed in a study of sorghum (Augé et al. 2007). These findings indicate that the relationship between root and soil AMF hyphal colonization may vary among plant species. Consequently, a comprehensive evaluation of soil hyphal density in *Piriqueta* would be needed to determine the contribution of soil-borne hyphae in promoting drought tolerance within this complex.

It is notable that plants grown with field soil from the more arid *caroliniana* region and other field soil treatments had similar biomass at harvest, despite low levels of AMF colonization (27.2% and 36.3% of total colonization levels in H and V region soils, respectively). This finding is paradoxical given what is known about the ecological function of AMF biomass in the soil environment (Tisdall 1994; Tarafdar 1995; Marschner and Timonen 2005). It is possible that the low levels of colonization among plants inoculated with soil from the *caroliniana* region are indicative of an AMF ecotype with a greater capacity to improve plant water status. Direct approaches to quantify water use efficiency in the *Piriqueta*-AMF system (e.g., $\delta^{13}\text{C}$ isotope analysis) would be required to make this determination, however. Another way in which AMF may have promoted drought tolerance was by mitigating osmotic stress in plants (reviewed in Ruiz-Lozano 2003), a mechanism found to operate independently of AMF-related

effects on host plant nutrient acquisition. Measurements of leaf osmotic potential and stomatal conductance in *Piriqueta* could help discern the means by which AMF colonization helps promote short-term drought tolerance observed in this plant species (Porcel and Ruiz-Lozano 2004).

Our findings suggest that plants of the V morphotype (which typically grows in the sandy, calcareous, low-phosphate, and periodically flooded soils of southern Florida) have evolved to be more reliant on AMF for growth under drought. Previous studies have concluded that nonmycorrhizal species typically evolve in phosphate-rich habitats where the cost of the symbiosis to the plant outweighed the benefits (Olsson and Tyler 2004). Consequently, plant species that have evolved in soils low in exchangeable phosphate may be expected to have a greater reliance upon AMF for their fitness and survival. These results suggest that while plants of the V morphotype may in fact be more reliant upon AMF colonization for growth under drought, this difference may ultimately be a consequence of having evolved in soils low in exchangeable phosphate.

Our findings raise the question of whether AMF obtained from more arid biomes (such as the *caroliniana* morphotype native habitat) could be generally better adapted at mitigating drought stress compared to their mesic counterparts. Plants inoculated with field-soil from the *caroliniana* region exhibited the greatest gains in biomass for both the C and V morphotypes. Although these improvements in shoot dry mass were not significantly greater than those observed with the other field soil treatments, the trend suggests that the AMF taxa associated with the different *Piriqueta* habitats may be divergent in their ecological functions (Allen et al. 1995). Current research in the field of mycorrhizal ecology underscores how little is known about relationships between AMF diversity and functionality (Parrent et al. 2010). Overall, the extent to which functional diversity exists between different strains of AMF is poorly understood (van der Heijden et al. 1998). Other researchers have advocated an approach emphasizing AMF-species assemblages (compared to studies investigating single-species scenarios) to help circumvent these knowledge gaps and help elucidate the roles of AMF in terrestrial ecology in biologically and ecologically relevant units (Gamper et al. 2010). More detailed research investigating the composition and function of the AMF assemblages occurring across the *Piriqueta* range would be required to verify whether the differences in drought tolerance

we observed were a result of differences in AMF species composition (e.g., Augé et al. 2003). While it is widely accepted that AMF species may differ in their functionality (Helgason et al. 2002; Gamper et al. 2010), it remains unclear if the drought tolerance observed in that study was the result of biotic interactions between different taxa of AMF (van der Heijden et al. 1998), soil ecological interactions with other classes of soil microbes or mutualists (Boucher et al. 1982), or possibly a unique AMF species particularly effective at promoting drought tolerance (Ruiz-Lozano et al. 1995).

Broader Implications

Our research supports other findings indicating that genetic differences among plant ecotypes may contribute to the outcome of mycorrhizal symbiosis (Johnson et al. 1997b; Wolfe et al. 2005). While a multitude of studies support the role of AMF in plant nutrition and soil fertility (reviewed in Jeffries et al. 2003; Smith and Read 2008), relatively little research has been performed to address the ecological and evolutionary consequences of AMF symbiosis in environments subject to drought (Hart et al. 2003; van der Heijden et al. 1998). In the case of *Piriqueta cistoides* spp. *caroliniana*, our research indicates that different ecotypes of AMF may have evolved among plant morphotypes associated with habitats differing in mineral nutrients and water availability. This suggests some potential for finding or developing more efficient strains of fungi for cultivating plants in water limited systems. Further research on AMF symbiosis in the *Piriqueta* complex and other drought-tolerant taxa may help elucidate how AMF-mediated responses may moderate the effects of drought on plant growth.

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