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Effects of arbuscular mycorrhizal fungi on seedling growth and development of two wetland plants, *Bidens frondosa* L., and *Eclipta prostrata* (L.) L., grown under three levels of water availability

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Abstract To identify the importance of arbuscular mycorrhizal fungi (AMF) colonizing wetland seedlings following flooding, we assessed the effects of AMF on seedling establishment of two pioneer species, *Bidens frondosa* and *Eclipta prostrata* grown under three levels of water availability and ask: (1) Do inoculated seedlings differ in growth and development from non-inoculated plants? (2) Are the effects of inoculation and degree of colonization dependent on water availability? (3) Do plant responses to inoculation differ between two closely related species? Inoculation had no detectable effects on shoot height, or plant biomass but did affect biomass partitioning and root morphology in a species-specific manner. Shoot/root ratios were significantly lower in non-inoculated *E. prostrata* plants compared with inoculated plants (0.381±0.066 vs. 0.683±0.132). Root length and surface area were greater in non-inoculated *E. prostrata* (259.55±33.78 cm vs. 194.64± 27.45 cm and 54.91±7.628 cm^2 vs. 46.26±6.8 cm^2, respectively). Inoculation had no detectable effect on *B. frondosa* root length, volume, or surface area. AMF associations formed at all levels of water availability. Hyphal, arbuscular, and vesicular colonization levels were greater in dry compared with intermediate and flooded treatments. Measures of mycorrhizal responsiveness were significantly depressed in *E. prostrata* compared with *B. frondosa* for total fresh weight (−0.3±0.18 g vs. 0.06±0.06 g), root length (−0.78±0.28 cm vs.−0.11±0.07 cm), root volume (−0.49± 0.22 cm^3 vs. 0.06±0.07 cm^3), and surface area (−0.59± 0.23 cm^2 vs.−0.03±0.08 cm^2). Given the disparity in species response to AMF inoculation, events that alter AMF prevalence in wetlands could significantly alter plant community structure by directly affecting seedling growth and development.

Keywords Arbuscular mycorrhizal fungi · Wetlands · Mycorrhizal responsiveness · Flooding · *Bidens frondosa* · *Eclipta prostrata*

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Introduction

While the effects of AMF on plant physiology (Auge 2001; Evelin et al. 2009; Smith et al. 2010), soil stability and nutrient cycling (Bethlenfalvay and Linderman 1992; Bethlenfalvay and Schüepp 1994; Jastrow and Miller 1991; Rillig and Mummey 2006), and plant community structure (Daleo et al. 2008; Escudero and Mendoza 2005; Jackson and Mason 1984; van der Heijden 1998) in terrestrial environments are well known, the importance of AMF in aquatic and wetland habitats has received little attention (Cornwell et al. 2001; Muthukumar et al. 2004; Stevens et al. 2002; Stevens and Peterson 2007; Turner and Friese 1998). Historically, AMF were thought to be absent or rare in wetland plants (Crawford 1992; Khan and Belik 1995; Khan 2004; Peat and Fitter 1993). In part, this was attributable to a perceived inability of AMF to survive anaerobic conditions found in reduced wetland soils and/or a decreased need for nutrient augmentation by AMF since plants could potentially acquire nutrients from water and the substrate across the leaf and root surfaces (Cooke et al. 1993; Peat and Fitter 1993). An increasing number of studies have, however, revealed that many wetland plant species harbor AMF and AMF have been found in wetland

habitats (Cooke and Lefor 1998) ranging from bottomland hardwood forest (Stevens et al. 2010), degraded Cypress swamps (Kandalepas et al. 2010), marshy environments (Bohrer et al. 2004; Radhika and Rodrigues 2007), groundwater-fed wetlands (Turner et al. 2000), freshwater fens (Bohrer et al. 2004; Cornwell et al. 2001; Šraj-Kržič et al. 2006), calcareous fens (Wolfe et al. 2006), and salt marshes (Brown and Bledsoe 1996; Carvalho et al. 2003). The prevalence of AMF in wetlands is now recognized; the dependency of wetland plants on their AMF partners and the factors that affect levels of AMF colonization in wetland habitats are poorly understood (Cornwell et al. 2001; Muthukumar et al. 2004; Stevens et al. 2002; Stevens and Peterson 2007; Turner and Friese 1998).

Wetlands are characterized by the presence of flooded or saturated soils for at least part of the growing season (Cowardin et al. 1979). Interspecific differences in the capacity to tolerate or avoid conditions associated with flooded or saturated soils (i.e., reduced soil oxygen availability, altered nutrient availability, and the build up of toxic ions) are a major determinant of wetland plant community structure (Keddy 2002; Mitsch and Gosselink 2000). While seasonal or episodic flooding maintain wetland plant community structure through elimination of less flood tolerant upland species (Middleton 1999), prolonged flooding can result in widespread vegetation loss. Following severe flooding and vegetation loss, wetland plant communities may reestablish through contributions from the soil seed bank (see Keddy 2002; van der Valk 1981). Several studies of terrestrial habitats suggest that plants established following disturbance tend to be non-mycorrhizal (see Reeves et al. 1979; Smith et al. 2010; Smith and Read 2002), however, Stevens et al. (2010) found AMF colonization in 31 plant species established following a prolonged flood in a remnant bottomland forest in north central Texas. While this implies a role of AMF in the reestablishment of wetland plant species following disturbance, experimental support is lacking.

The seedling stage is the most vulnerable stage in a plant's life cycle (Grubb 1977), and responses to flooding at the seedling stage are considered one of the most important determinants of species composition in bottomland swamps (Bedinger 1978), and possibly other types of wetlands (Middleton 1999). While understanding the factors affecting seedling establishment and survival are crucial for wetland restoration and management (Keddy 2002; Middleton 1999), as well as community/population diversity and dynamics (Grime and Hiller 1992), little is known of the responses of seedlings to various hydrologic treatments (Fraser and Feinstein 2005), and we are unaware of any studies that have evaluated the effects of AMF on wetland seedlings. Previous studies that have sought to quantify effects of AMF on wetland plant growth and identify interaction effects of AMF and water availability have examined effects on established plants (see Carvalho et al. 2003; Garcia et al. 2008; Ipsilantis and Sylvia 2007; Miller 2000; Miller and Sharitz 2000; Osundina 1998; Šraj-Kržič et al. 2006; Stevens and Peterson 1996; Stevens and Peterson 2007; Wolfe et al. 2006). Consequently, several questions remain regarding the role of AMF in early seedling establishment in wetlands and the conditions required for AM colonization of wetland seedlings. While there is a general trend towards a reduction in AM colonization with increasing water availability (Escudero and Mendoza 2005; Miller 2000; Osundina 1998; Stevens and Peterson 1996), the high incidence of AM colonization in seedlings established immediately after a 100-year flood in a remnant bottomland hardwood forest suggests that colonization can occur rapidly and possibly under wet conditions (Stevens et al. 2010).

To identify the importance of AMF colonizing wetland plants following flooding, we assess the effects of AMF on seedling establishment of two pioneer species, *Bidens frondosa* L. and *Eclipta prostrata* (L.) L. grown under three levels of water availability. We ask the following:

1. Do inoculated seedlings differ in their growth and development from non-inoculated plants?
2. Are the effects of inoculation and degree of colonization dependent on water availability?
3. Do plant responses to inoculation differ between two closely related species?

Materials and methods

Experimental design

The experiment was a 2×2×3 randomized complete block design with two wetland plant species (*B. frondosa* L. and *E. prostrata* (L.) L.), two AMF treatments (inoculated and non-inoculated), and three levels of water availability (water levels maintained at the soil surface, 3.5 cm below the soil surface, and no-standing water but watered twice daily). Individually potted seedlings were placed in 29.3 L (67.8×40.1×17.5 cm) plastic trays and grown on shelves in a growth room at the University of North Texas. A total of 12 plants were grown in each tray (two species×two levels of AMF×three subsamples (plants)/tray); all plants were randomized within trays and all trays were randomly assigned a position and treatment within shelves. Each of the five shelves used constituted one block and contained one tray for each of the three water level treatments. Shelves were lit by a bank of eight high intensity

fluorescent lights (Sun System Tek Light T-5 high output fluorescent fixture with three VitaLume Plus Bloom and three VitaLUME Plus Grow bulbs) providing an average of 459 μmols/m^2 PAR on 16/8-light/dark cycle. Temperature was maintained at 23°C.

Establishment of AMF cultures

Mycorrhizal cultures were established using riparian soils obtained from the Elm Fork of the Trinity River, Denton, Texas. Five 5-gallon buckets of soil were obtained and spores extracted following the methods described by Brundrett et al. (1996). Five trays (60× 30×15 cm) were filled with locally obtained masonry sand to which the isolated, washed spores were added. Three native, locally abundant wetland species (*B. frondosa*, *E. prostrata* and *Sesbania herbacea* (Mill.) McVaugh) were germinated in Petri dishes on the surface of moist filter paper then transplanted to trays. Cultures were maintained under growth room conditions. To prepare the inoculum, seedlings were uprooted from the culture trays, the roots excised, washed, and blended to obtain a slurry.

Seedling establishment

Seeds of B. *frondosa* and *E. prostrata* were collected in the fall of 2007 from the floodplain of the Elm Fork of the Trinity River and stored at room temperature. Seeds were germinated on the surface of moist filter paper in sealed 15 cm Petri dishes under growth room conditions. Germination began within 2 days, and after 5 days the germinated seeds were transplanted into an 8×8×9 cm plastic pots. Pots were filled with masonry sand and for the inoculated treatments, 15 ml of inoculum was added to a small well made in the sand at the center of the pot. Each pot was internally lined with a piece of Whatman # 41 filter paper to retain the sand and prevent cross-contamination. For the two wettest treatments, a 6 mm stand-pipe was used in each tray to maintain water levels at the soil surface and 3.5 cm below the soil surface. A Manostat Carter Multi-Channel Precision 12/6 cassette pump (Cole-Parmer Instrument Co., Vernon Hills, IL) maintained a continuous flow of 1/64 Long Ashton's nutrient solution (Hewitt 1966)—delivering approximately 6.4 mg/l of phosphorous—at an average flow of 0.085 L/h for the intermediate and wet microcosms. Nutrient solution was made up in a 70 L reservoir and refilled every 48 h. For the driest treatment, plants were watered twice daily with 25 ml of 1/64 strength Long Ashton nutrient solution. Prior studies indicate that this level of nutrient availability is sufficient to maintain plant growth without inhibiting AM colonization (Stevens et al. 2002; White and Charvat 1999).

Harvesting and assessment

Harvesting began 50 days after seedlings were transplanted and continued for a 48-h period, with each block being harvested within a 2-h period. Stems were removed at the soil surface and main stem height and fresh weight was recorded. Stems were bagged and dried at 40°C for dry mass determination. Roots were freed of the soil substrate by gentle agitation of the root system under water. To prevent root loss water was filtered through 250 μm sieve and any severed roots collected. Root fresh weight was determined and the root system was digitized using an Epson Expression 10000 XL color photo scanner at 400 dpi. After scanning, roots were stored in 50% ethanol. Root length, volume and surface area were determined using WinRHIZO PRO (ver 2007c Regent Instruments, Quebec, Canada). A subsample of non-woody lateral roots was obtained for determination of AMF colonization levels. Roots were cleared by autoclaving in 10% potassium hydroxide for 20 min and then stained with 0.1% Chlorazol Black E for 40 min in an autoclave at 121°C (Brundrett et al. 1996). Roots were destained and stored in 50% glycerol prior to mounting on slides in 50% glycerol (Phillips and Hayman 1970). Slides were viewed with 200× magnification using a Zeiss Axio image microscope and images obtained with a Zeiss Axiocam MRC-5 camera. Colonization levels were assessed using a modified grid line intersect procedure (McGonigle et al. 1990). A total of 100 fields of view were assessed for each sample.

Data analysis

Plant growth responses were analyzed using a three-way analysis of variance (ANOVA) in SAS 9.1 (SAS Institute Cary, NC), with species, water availability and AMF colonization as main effects and species x water availability, species x AMF colonization, water availability x AMF colonization and species x water availability x AMF colonization as interaction effects. Blocks and subsamples within blocks were treated as random effects. To meet requirements of normality and equal variance shoot height, shoot fresh and dry weight, root length, surface area and volume were log transformed, root fresh weight was square root transformed and shoot/root (S/R) fresh weight ratios were analyzed using ranked data. AMF colonization levels were analyzed using a two-way ANOVA in SAS with species and water availability as main effects and species x water availability as the interaction effect. To meet assumptions of normality and equal variance analyses were conducted on ranked data. When significant main effects or interaction effects were detected, multiple comparisons were conducted using the Tukey–Kramer option in SAS.

Mycorrhizal responsiveness (MR) was assessed as the difference in morphometric characteristics of inoculated and non-inoculated plants relativized through division by the response of inoculated plants (Janos 2007). Since there were three plants for each treatment combination in each block, MR was calculated for each species at each level of water availability in each block. MR was assessed for shoot height, shoot fresh and dry weight, root and total fresh weight, root length, volume and surface area. MR was analyzed using a two-way ANOVA with species and water availability as main effects and species x water availability as interaction effect. When significant main effects or interaction effects were detected, multiple comparisons were conducted using the Tukey–Kramer option in SAS. For all figures untransformed means are presented ±1 standard error.

Results

Shoot height was affected by water availability, species and the water availability x species interaction (Table 1). Shoot height was consistently higher for *B. frondosa* compared with *E. prostrata* at all levels of water availability (Fig. 1) and for both species was significantly higher in the dry treatment compared with the intermediate and flooded treatment. Shoot fresh and dry weight, root fresh weight and total fresh weight were affected by water availability, species and the water availability x species interaction (Table 1). While there were no significant differences in shoot fresh weight, root fresh weight or total fresh weight between species in the dry treatment (Fig. 2a–c), all were significantly lower in *E. prostrata* compared with *B. frondosa* in the intermediate and wet treatments. For both species, shoot fresh weight and root fresh weight were significantly higher in the dry compared with the intermediate and wet treatments. Shoot dry weight was significantly lower in the intermediate and wet treatments compared with the dry treatments for both *E. prostrata* and *B. frondosa*, and was significantly lower in *E. prostrata* compared with *B. frondosa* at all levels of water availability (Fig. 2d).

Shoot/root fresh weight ratio (S/R) differed among species, inoculation (AMF), and was affected by the interaction of water availability x species and species x AMF (Table 1). S/R was lower in *E. prostrata* compared with *B. frondosa* in the dry treatment but did not differ among species in the intermediate and wet treatments (Fig. 3). Within species, S/R was significantly lower in the dry treatment compared with the wet treatment for *B.*

Table 1 Summary table of three-way ANOVA assessing the effects of water availability (Water) and AMF inoculation (AMF) on the growth of *Eclipta prostrata* and *Bidens frondosa*

Response variable	Water		Sp		AMF		Water×Sp		Water×AMF		Sp×AMF		Water×Sp×AMF	
	F	*p* Value	F	*p* Value	F	*p* Value	F	*p* Value	F	*p* Value	F	*p* Value	F	*p* Value
Shoot height	55.97	<.0001	437.77	<.0001	0.97	0.3252	12.76	<.0001	0.24	0.7864	0.52	0.4732	0.66	0.5158
Shoot fresh weight	115.81	<.0001	115.71	<.0001	1.33	0.2498	12.91	<.0001	0.28	0.7571	0.41	0.5205	0.63	0.5316
Shoot dry weight	132.58	<.0001	162.27	<.0001	1.92	0.1681	11.08	<.0001	0.53	0.5870	2.27	0.1341	1.30	0.2745
Root fresh weight	114.24	<.0001	46.37	<.0001	0.60	0.4397	8.92	0.0002	0.63	0.5351	1.10	0.2949	0.14	0.8720
Shoot/root fresh weight	2.61	0.0764	5.39	0.0215	9.71	0.0022	7.11	0.0011	1.68	0.1901	9.65	0.0022	1.52	0.2224
Total fresh weight	120.23	<.0001	57.62	<.0001	0.00	0.9619	6.92	0.0013	0.24	0.7880	1.12	0.2919	0.11	0.8993
Root length	55.70	<.0001	30.34	<.0001	9.24	0.0028	13.37	<.0001	0.45	0.6391	4.33	0.0391	0.31	0.7324
Root volume	98.50	<.0001	59.21	<.0001	1.71	0.1927	11.31	<.0001	1.28	0.2799	5.12	0.0250	0.18	0.8362
Root surface area	76.77	<.0001	40.45	<.0001	4.51	0.0352	13.28	<.0001	1.18	0.3115	4.43	0.0369	0.16	0.8537

Significant effects (p=<0.05) are in bold

Sp species

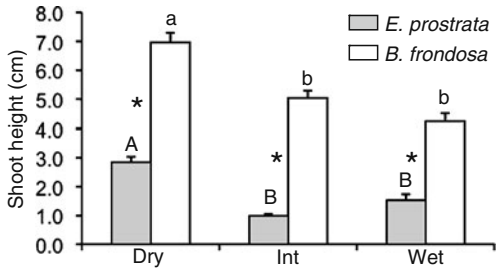

Fig. 1 Effect of water availability on shoot height of *Eclipta prostrata* and *Bidens frondosa* grown under three levels of water availability (Dry, intermediate (Int), and Wet). Different *uppercase letters* indicate significant differences ($p<0.05$) among *E. prostrata* plants grown across levels of water availability. Different *lowercase letters* indicate significant differences among *B. frondosa* plants grown across levels of water availability. *Asterisk* indicate significant differences between species within levels of water availability. Raw means are presented with *bars* indicating standard error

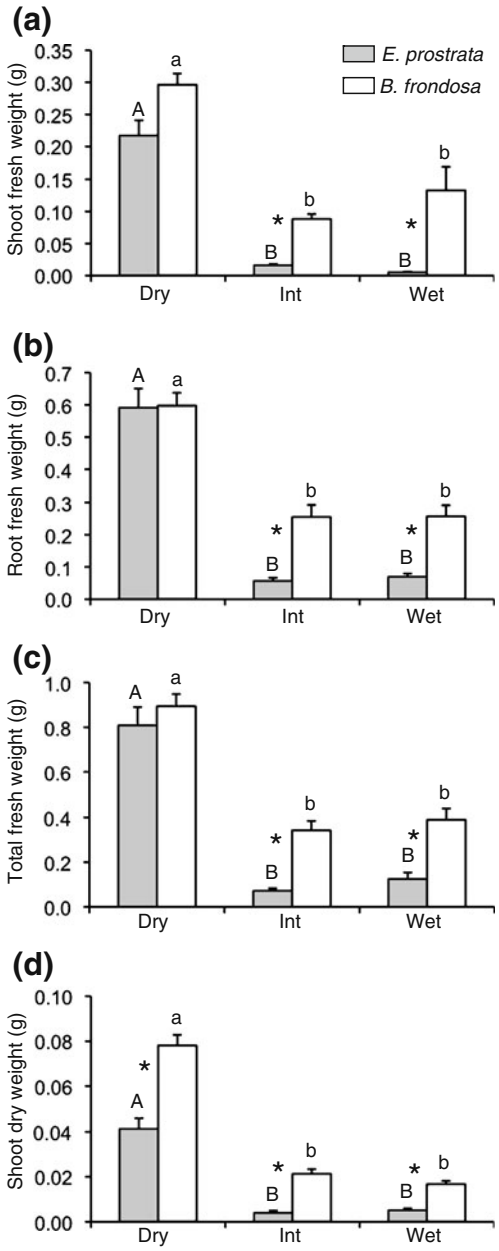

Fig. 2 Effect of water availability on shoot fresh weight (**a**), root fresh weight (**b**), total fresh weight (**c**), and shoot dry weight (**d**) of *Eclipta prostrata* and *Bidens frondosa* grown under three levels of water availability (Dry, intermediate (Int), and Wet). Different *uppercase letters* indicate significant differences ($p<0.05$) among *E. prostrata* plants grown across levels of water availability. Different *lowercase letters* indicate significant differences among *B. frondosa* plants grown across levels of water availability. *Asterisk* indicate significant differences between species within levels of water availability. Raw means are presented with *bars* indicating standard error

frondosa. Although there were no significant differences in S/R among species in inoculated plants, in non-inoculated plants S/R was significantly lower in *E. prostrata* compared with *B. frondosa* (Fig. 3). For *E. prostrata* S/R was significantly lower in non-inoculated compared with inoculated plants.

Root length, volume and surface area differed among species, AMF, and were affected by the interactions of water availability x species and species x AMF (Table 1). While there were no significant differences in root length, volume and surface area between species in the dry treatment, these were significantly greater in *B. frondosa* in the intermediate and wet treatments compared with *E. prostrata* (Fig. 4a–c). For both species root length, volume and surface area was significantly greater in the dry treatment compared with the intermediate and wet treatment (Fig. 4a–c). There were no detectable effects of inoculation on root length, volume and surface area of *B. frondosa* (Fig. 4a–c), however, root length and surface area were greater in non-inoculated compared with inoculated treatments for *E. prostrata*. With the exception of root length in the non-inoculated plants, root length, surface area and volume were significantly greater in *B. frondosa* compared with *E. prostrata*.

Hyphal, vesicular and arbuscular colonization levels were affected by water availability, however, there were no significant differences attributable to interspecific responses or the interaction of species x water availability for any measure of colonization (Table 2). All three measures of colonization were significantly greater in the dry treatment compared with the intermediate and dry treatments with no significant differences between intermediate and wet treatments (Fig. 5). There were no significant effects of water availability, species or the interaction of species x water availability on mycorrhizal responsiveness (MR) for shoot height, shoot fresh and dry weight and root fresh weight (Table 3; Fig. 6). The MR of each species differed for total fresh weight, root length, volume and surface area, however MR was not significantly affected by

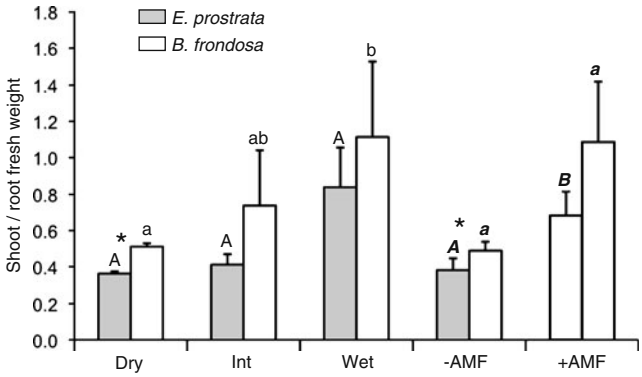

Fig. 3 Effect of water availability and AMF inoculation on shoot/root fresh weight ratio of *Eclipta prostrata* and *Bidens frondosa* grown under three levels of water availability (Dry, intermediate (Int), and Wet). Different *uppercase letters* indicate significant differences ($p<0.05$) among *E. prostrata* plants grown across levels of water availability. Different *lowercase letters* indicate significant differences among *B. frondosa* plants grown across levels of water availability. Bold and italics letters indicate differences in AMF inoculation. *Asterisk* indicate significant differences between species within levels of water availability and inoculation. Raw means are presented with *bars* indicating standard error

water availability or the interaction of species x water availability (Table 3). Total fresh weight, root length, volume and surface area were significantly depressed in *E. prostrata* compared with *B. frondosa* (Fig. 6).

Discussion

Reductions in AMF colonization levels with increasing levels of water availability are consistent with previous field and greenhouse/growth-room studies (Stevens and Peterson 1996; Rickerl et al. 1994). Water availability is not the sole factor affecting AMF colonization levels in wetland plants, however. Stevens and Peterson (2007) found that phosphorus availability and not water availability led to reduced levels of AMF colonization in the amphibious species *Lythrum salicaria*, while Carvalho et al. (2003) found that salinity had a greater effect on colonization of *Aster tripolium* L. than flooding. Furthermore, seasonal variability in colonization levels attributable to species-specific differences in phenology have been found in salt marsh (Carvalho et al. 2001), fen and fresh water marsh plants (Bohrer et al. 2004). While AMF colonization levels may be reduced in flooded soils compared with non-flooded soils, colonization levels of *E. prostrata* and *B. frondosa* in our flooded treatments were relatively high (<20%) compared with colonization in comparable treatments of other wetland plant species (i.e., <6% in *Typha latifolia* L., Ipsilantis and Sylvia 2007) but comparable to levels found in continuously flooded *A. tripolium*, a member of the same family as *E. prostrata* and *B. frondosa*.

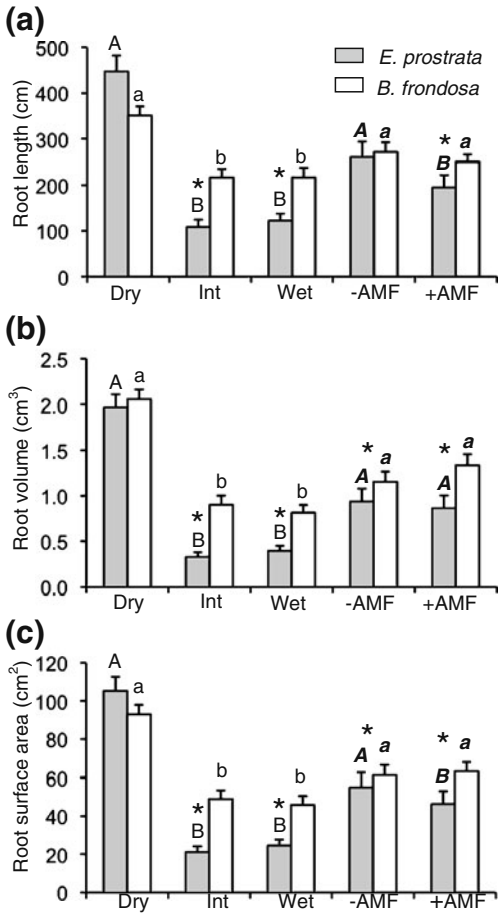

Fig. 4 Effect of water availability and AMF inoculation on root length (**a**), root volume (**b**), and root surface area (**c**) of *Eclipta prostrata* and *Bidens frondosa* grown under three levels of water availability (Dry, intermediate (Int), and Wet). Different uppercase letters indicate significant differences ($p<0.05$) among *E. prostrata* plants grown across levels of water availability. Different lowercase letters indicate significant differences among *B. frondosa* plants grown across levels of water availability. *Bold and italics letters* indicate differences in AMF inoculation. *Asterisk* indicate significant differences between species within levels of water availability or AMF inoculation. Raw means are presented with *bars* indicating standard error

The survival of non-inoculated *E. prostrata* and *B. frondosa* plants indicates that these are facultative mycorrhizal species.

AMF colonization has been documented in flooded roots of several emergent wetland plant species (i.e., Bagyaraj et al. 1979; Stevens and Peterson 1996; Weishampel 2005; Šraj-Kržič et al. 2006), yet what is not clear from these studies is *when* initial colonization occurred. In field studies it is often not possible to discern between colonization occurring when soils were flooded or during drawdown (Stevens and Peterson 1996; Ray and Inouye 2006). In greenhouse/growth room studies inoculation is often followed by a pretreatment period to facilitate AMF coloni-

Table 2 Summary table of two-way ANOVA assessing the effects of water availability (Water) on levels of AMF colonization of *Eclipta prostrata* and *Bidens frondosa*

Response variable	Water		Sp		Water×Sp	
	F	*p* Value	F	*p* Value	F	*p* Value
Hyphal colonization	16.01	<.0001	0.23	0.6326	2.15	0.1227
Vesicular colonization	19.59	<.0001	1.04	0.3117	2.80	0.0666
Arbuscular colonization	10.26	0.0001	2.02	0.1592	2.22	0.1153

Significant effects (p=<0.05) are in bold

Sp species

zation prior to water level manipulation (i.e., Garcia et al. 2008; Ipsilantis and Sylvia 2007). Consequently, while the effects of water availability on AMF levels have been documented *after* initial colonization has occurred, there are few studies that determine *if* colonization can occur under conditions of excess water availability. Miller and Sharitz (2000), utilizing vegetative propagules of *Panicum hemitomon* Schult. and *Leersia hexandra* Sw., exposed plants to soil inundation immediately following AMF. They found soil inundation inhibited AMF establishment and exposure to constant soil inundation resulted in total colonization levels near zero. Carvalho et al. (2003) transplanted 4-week-old *A. tripolium* plants to field collected soils and imposed three levels of water availability. Total colonization levels in their continuously flooded treatments conditions were significantly lower than in pulsed or drier treatments, however, they were quite high (>20%) compared with the results of Miller and Sharitz (2000) and comparable to the results obtained in the current study with *E. prostrata* and *B. frondosa*. Although methodologies differ among studies by Miller and Sharitz (2000), Carvalho et al. (2003) and the current study, together they indicate that while soil inundation may inhibit AMF formation in some emergent wetland species under certain conditions, this is not always the case and AMF associations can establish in inundated soils.

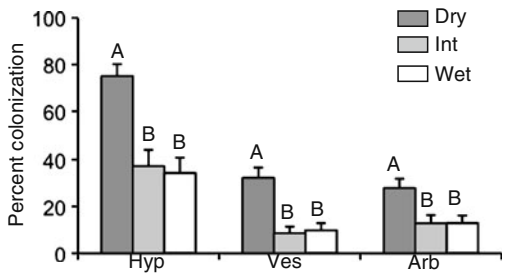

Fig. 5 Effect of water availability on arbuscular mycorrhizal colonization levels on plants grown under three levels of water availability (Dry, intermediate (Int), and Wet). Different *uppercase letters* indicate significant differences (p<0.05) within each classification of AMF colonization (*Hyp* hyphal, *Ves* vesicular, *Arb* arbuscular colonization). Pooled raw means of *E. prostrata* and *B. frondosa* plants are presented±standard error

Regardless of water availability we were not able to detect a significant effect of AMF inoculation on shoot height or biomass of *B. frondosa* or *E. prostrata*. The documented effects of AMF inoculation on wetland plant performance are, however, inconsistent. Whereas an increase in aboveground measures of plant performance were found in inoculated *Carex tribuloides* Wahlenb., *Phalaris arundinacea* L., and *Rumex orbiculatus* A. Gray (Fraser and Feinstein 2005), *Casuarina equisetifolia* L. (Osundina 1998), *P. hemitomon*, and *T. latifolia* (Dunham et al. 2003), a reduction in aboveground measures of plant performance was found in inoculated *L. salicaria* plants (Stevens et al. 2002; Stevens and Peterson 2007). Shoot height and biomass are often used as surrogates of fitness, though an absence of a significant effect on shoot height and biomass does not imply an absence of a fitness contribution. Field grown plants interact with their biotic and abiotic environments in complex ways, therefore any potential contribution to fitness may only manifest when assessed under a broader range of conditions (Smith et al. 2010).

Since AMF are generally able to forage for resources more economically than host plant roots, colonized plants tend to invest fewer resources in root system development compared with non-colonized plants, and a trend towards an increase in S/R ratios and reduced root biomass has been found in inoculated terrestrial plants (Smith and Read 2002). Although similar responses have been shown to occur in wetland plant species (Fraser and Feinstein 2005; Cerligione et al. 1988; Neto et al. 2006), White and Charvat (1999) found a reduced S/R ratio in inoculated *L. salicaria* plants. In the current study, AMF inoculation was associated with an increase in S/R fresh weight in *E. prostrata* but not *B. frondosa*, yet there were no significant effects on root biomass. Root morphology also differed between species in response to AMF inoculation; whereas inoculation resulted in reduced root length and surface area for *E. prostrata*, there were no significant differences in these parameters in inoculated and non-inoculated *B. frondosa* plants. In contrast,

Table 3 Summary table of two-way ANOVA assessing the effects of water availability (Water) on mycorrhizal responsiveness (MR) of *Eclipta prostrata* and *Bidens frondosa*

Response variable	Water		Sp		Water x Sp	
	F	*p* Value	F	*p* Value	F	*p* Value
Shoot height	0.290	0.7536	0.04	0.840	1.10	0.3509
Shoot fresh weight	0.670	0.5213	0.01	0.905	0.62	0.5471
Shoot dry weight	0.100	0.9085	0.02	0.898	1.37	0.2776
Root fresh weight	0.770	0.4763	2.03	0.170	0.79	0.4676
Total fresh weight	0.040	0.9564	4.54	0.046	0.31	0.7335
Root length	0.003	0.0968	5.58	0.029	0.29	0.7517
Root volume	0.720	0.4993	5.98	0.024	0.53	0.5992
Root surface area	0.420	0.6610	5.31	0.032	0.60	0.5565

Significant effects (p=<0.05) are in bold

Sp species

a trend towards increased root length in inoculated *L. hexandra* and *P. hemitomon* plants was found by Miller and Sharitz (2000), while no effect of inoculation on root length or root surface area of *L. salicaria* was found by Stevens et al. (2002). It must be emphasized that the preceding studies differed in many respects (i.e., plant species, flooding duration, frequency and depth of flooding, nutrient availability, study duration), however, the results suggest site and species-specific differences in root system morphology and biomass portioning in response to AMF inoculation.

Mycorrhizal responsiveness, as defined by Janos (2007) is "the difference in growth between mycorrhizal and non-mycorrhizal plants at a designated level of phosphorus availability" and can be relativized by expression in terms of growth of either inoculated or non-inoculated plants. In this study, total productivity, measured as total fresh weight, and root morphology were more responsive to AMF colonization in *E. prostrata* compared with *B. frondosa*. Whereas productivity and root morphology of *B. frondosa* showed little response to inoculation, a negative response was displayed in *E. prostrata*. These results should not, however, be construed as indicating differences in host species intrinsic capabilities to respond to various mycorrhizal fungal species (Janos 2007), nor should they be interpreted as indicating that overall fitness of colonized *E. prostrata* plants was at a disadvantage (Smith et al. 2010); such conclusions require assessments conducted over a much broader range of conditions. Our results reveal differences in mycorrhizal responsiveness among two sympatric wetland Asteraceae. Although once thought to be absent in wetlands, AMF have now been found in many diverse wetland types (Stevens et al. 2010; Kandalepas et al. 2010; Radhika and Rodrigues 2007). Furthermore, while their role in secondary succession has been thought to be minimal, Stevens et al. (2010) documented widespread colonization in 31 out of 37 wetland plant species establishing in a bottomland hardwood forest following prolonged flooding. This study has shown that AMF can colonize plants at a very early stage in their development across a wide range of water availabilities—including inundated soils—and have the capacity to affect patterns of resource allocation and root morphology that is species and environment specific. The study also shows that two closely related wetland species both in the Asteraceae differ in mycorrhizal responsiveness. If a wide disparity in mycorrhizal responsiveness among seedlings of wetland plant species exists, as has been found in some upland species (i.e., Janos 1980; Saif 1987), then it could be expected that events that alter AMF prevalence in wetlands could significantly alter plant community structure by directly affecting seedling growth and development and the interactions of seedlings with other organisms. Since wetland plant seedlings are generally more responsive to hydrology than adult plants (Bedinger 1978), understanding interactions among AMF, seedling growth and development, and hydrology in wetlands may provide greater insight into the factors shaping wetland plant community structure.

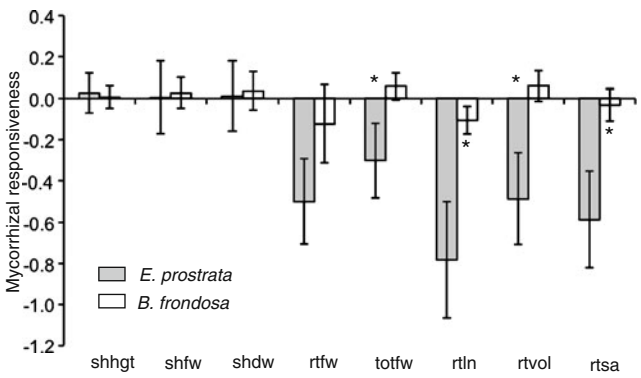

Fig. 6 Comparisons of mycorrhizal responsiveness of *E. prostrata* and *B. frondosa* grown at three levels of water availability. *Shhgt* shoot height, *shfw* shoot fresh weight, *shdw* shoot dry weight, *rtfw* root fresh weight, *totfw* total fresh weight, *rtln* root length, *rtvol* root volume and *rtsa* root surface area. *Asterisk* indicate significant differences (p<0.05) between species. Pooled means for all levels of water availability for each species are presented±standard error

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