10-1-2009

# Sex-Specific Variation in the Interaction Between Distichlis Spicata (Poaceae) and Mycorrhizal Fungi

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Citation Details

Eppley, S. M, Mercer, C. A., Haaning, C., Graves, C. B. 2009. Sex-specific variation in the interaction between Distichlis spicata (Poaceae) and mycorrhizal fungi. American Journal of Botany 96: 1967-1973

American Journal of Botany 96(11): 1967–1973. 2009.

# SEX-SPECIFIC VARIATION IN THE INTERACTION BETWEEN *DISTICHLIS SPICATA* (POACEAE) AND MYCORRHIZAL FUNGI[1]

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Associations between mycorrhizal fungi and plants can influence intraspecific competition and shape plant population structure. While variation in plant genotypes is known to affect mycorrhizal colonization in crop systems, little is known about how genotypes affect colonization in natural plant populations or how plant sex might influence colonization with mycorrhizal fungi in plant species with dimorphic sexual systems. In this study, we analyzed mycorrhizal colonization in males and females of the wetland dioecious grass *Distichlis spicata*, which has spatially segregated sexes. Our results suggest that *D. spicata* males and females interact with mycorrhizal fungi differently. We discuss the implications for the role of this sex-specific symbiotic interaction in the maintenance of the within-population sex ratio bias of *D. spicata*.

**Key words:** dioecy; *Distichlis spicata*; mycorrhizal fungi; plant; Poaceae; sex ratio; sexual dimorphism; symbiosis.

Mycorrhizal fungi form symbiotic relationships with the vast majority of flowering plants (Newman and Reddell, 1987), and while the relationship has generally been interpreted to be mutualistic, it may vary along a continuum from mutualism to parasitism (Johnson et al., 1997). The benefit for fungi in this symbiosis is primarily direct carbohydrate gain from plant hosts (Finlay and Söderström, 1992), while the benefit for plants is more complex and may include increased uptake of limited nutrients (Marschner and Dell, 1994), as well as protection from pathogenic root fungi (Newsham et al., 1995), protection from drought stress (Allen and Allen, 1986), and improved soil structure (Miller and Jastrow, 2000). As a result of the effects of this symbiosis on plant fitness, mycorrhizal fungi can influence plant population structure (e.g., Eissenstat and Newman, 1990; Allsopp and Stock, 1992). In particular, because mycorrhizal relationships increase nutrient access in nutrient poor soils, mycorrhizal associations have been found to increase intraspecific competition (e.g., Facelli et al., 1999; Pietikäinen and Kytöviita, 2007). Mycorrhizal interactions also generally increase size inequalities among plants within species (Moora and Zobel, 1998) and thus reproductive inequalities (Shumway and Koide, 1995). However, the role of mycorrhizal fungi in shaping plant populations is expected to be more complex than purely a density-dependent phenomenon because interactions between mycorrhizal fungi and plants are expected to vary depending on plant genotype (e.g., Azcon and Ocampo, 1981; Heckman and Angle, 1987; Yao et al., 2001; Singh et al., 2002). Data on the variation in mycorrhizal colonization with genotype currently comes from crop systems (reviewed by Smith and Goodman, 1999), but natural populations are expected to have a similar pattern.

Differential response by male and female plants to mycorrhizal infection is one genotype-specific aspect of mycorrhizal–plant relationships that has been examined, but for which evidence has not been previous found (Gehring and Whitham, 1992; Varga and Kytöviita, 2008). However, dioecious species with biased sex ratios have not been examined as far as we are aware. Sex-specific interactions between angiosperms and mycorrhizal fungi could have significant impacts on plant population sex ratios, plant population structures, and the coevolution of the fungus–plant relationship. Sexual dimorphism—differences between male and female individuals in primary and secondary sexual characters—is thought to have evolved in higher plants due to sex-specific selection in which males and females experience different selection pressures (Smouse and Meagher, 1994). Over evolutionary time, this mode of selection leads to differentiation of males and females of the same species in morphological, physiological, and life-history traits (see Dawson and Geber, 1999; Delph, 1999; Eckhart, 1999 for reviews). Sex-specific differences in morphology, physiology, and life-history are likely to lead to differences in how males and females interact with other organisms and such sex-specific biotic interactions, including variation in responses to herbivory, parasitism, and competition, have been measured in a number of plant species and have the potential to influence sex ratios (Ågren et al., 1999).

In the present study, we analyzed mycorrhizal colonization in roots of male and female *Distichlis spicata* plants grown in natural populations and in the greenhouse to address the following questions: (1) Does sex-specific mycorrhizal colonization occur in reproductively mature plants? (2) Does sex-specific mycorrhizal colonization occur before plants research reproductive maturity? (3) Does sex-specific mycorrhizal colonization vary seasonally in natural populations?

*Distichlis spicata* is an ideal model system for our study. First, *D. spicata* has spatially segregated sexes (SSS) within a population (Freeman et al., 1976; Eppley et al., 1998). Habitats in the marsh that have lower nutrient concentrations have adult sex ratios near 100% male, and with higher nutrient concentrations have adult sex ratios near 100% female (Eppley, 2000, 2001). The sex ratio bias found within populations occurs for both ramets and genets, regardless of flowering status (Eppley et al., 1998). Consequently, the sex ratio bias found in *D. spicata*

[1] Manuscript received 6 March 2009; revision accepted 10 August 2009.

The authors thank A. Brown, C. Carpenter, T. Cheeke, G. Herdman, M. Kariminaser, O. Merritt, K. Leonard, B. Pace, S. Rogers, and E. Shortlidge for help in the laboratory. The authors thank A. Brown and two anonymous reviewers for valuable comments on earlier drafts of this manuscript. Research was supported by the National Science Foundation (DEB 0743461 to S.E.).

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doi:10.3732/ajb.0900076

has the potential to be influenced by sex-specific biotic interactions. Second, there are sex-specific differences in seed germination, seedling survival and seedling competitive effects in this species (Eppley, 2000, 2001, 2006), suggesting that sex-specific differences occur at early stages in this species. Third, previous intersexual competition experiments in *D. spicata* suggest that sex-specific biotic interactions might occur (Eppley, 2006), *D. spicata* is known to be colonized by arbuscular mycorrhizal fungi (AMF) (Allen and Cunningham, 1983; Cooke and Lefor, 1990; Hoefnagels et al., 1993; Johnson-Green et al., 1995), and AMF are known to have significant effects on measures of fitness in *D. spicata* (Allen and Cunningham, 1983). Fourth, a sex-linked marker is available to determine the sex of prereproductive individuals (Eppley et al., 2009).

## MATERIALS AND METHODS

***The study system and population sex ratios***—*Distichlis spicata* (L.) E. Greene (Poaceae) is a dioecious, perennial grass, that is common at high densities in estuaries on the east and west coasts of North America (Hitchcock, 1971). Pollen is dispersed by wind (Hitchcock, 1971), seeds are water-dispersed (S. Eppley, personal observations), and extensive asexual propagation occurs via rhizomes and tillers (Hitchcock, 1971). Field sites were located at Limantour Estuary in Pt. Reyes National Seashore, Marin County, California and Sand Lake estuary in Sand Lake County Park, near Tillamook, Oregon. Spatial segregation of the sexes had been previously determined for the Pt. Reyes population (Eppley et al., 1998). For the Sand Lake population, we established permanent plots and determined population sex ratio. Within a *D. spicata*-dominated salt marsh (roughly 2000 m × 200 m), 30 random males and 30 random females were marked using a random number generator and a topographic map, and the sex ratio was determined around each plant following Eppley et al. (1998). Global positioning system coordinates were taken at sites for mapping purposes. To map sex ratios, we used the program ArcGIS 9.2 (ESRI, Redlands, California, USA). Using the Geocoding tools in the program, we assigned addresses to data points, which mapped plots on transect lines. In the Spatial Analyst extension to ESRI ArcGIS, the Krigging tool was used to interpolate data between points to produce cell-based raster maps. This approach provided maps that allowed us to explore and illustrate trends in sex ratios in the salt marsh.

***Field collection of roots 2007***—To determine whether AMF colonization was sex-specific, we collected adults from the Sand Lake and Pt. Reyes populations of *D. spicata* in 2007. From the Oregon population, on 8 August 2007, we collected roots from nine males and nine females that were flowering at the time of harvest. From the California population on 14 July 2007, we collected roots from 10 males and 10 females that were flowering at the time of harvest. In both populations, we collected from plants spaced at least 5 m apart to increase the likelihood that individuals were not from the same genet. To collect roots for each sampled plant, we excavated roots and rhizomes to a depth of 0.25 m (>95% of *D. spicata* roots are in this zone within these marshes; S. Eppley, personal observation). We collected roots in a 2.5 cm radius surrounding each plant. Roots were collected in the field, kept on ice, and brought back to Portland State University where they were stored at 4°C for mycorrhizal analysis (described later).

***Soil collection for spore extraction***—Because site-specific differences in mycorrhizal abundance might influence AMF colonization in the males and females, we quantified mycorrhizal spore number in majority-female and majority-male sites. On 8 August 2007, soil was collected at six majority-female and seven majority-male permanent sites at Sand Lake, and spores were extracted and quantified following McKenney and Lindsey (1987).

***Field collection of roots 2008***—To determine how AMF colonization in male and female *D. spicata* varies seasonally, we collected roots from the population in the Sand Lake estuary during two time periods. We chose times during the summer and early fall, as these periods have been shown to be most active for AMF colonization in salt marsh systems (van Duin et al., 1990; Carvalho et al., 2001). Eight permanent plots were established, four in randomly chosen female-majority sites and four in randomly chosen male-majority sites, previous determined as described earlier). On 2 July, when plants were green but not flowering, the first collection of roots was made. Roots from eight plants were collected at least 1 m apart at each of the four male-majority plots and at each of the four female-majority plots. Roots were collected in the field as described, brought back to Portland State University where they were stored at 4°C for mycorrhizal analysis (see later). A second collection was made again at these permanent plots on 21 August, when females had set seeds and both males and females had finished flowering.

***Soil samples 2008***—In October 2008, to determine how majority-female and majority-male sites different in soil nutrient availability, we collected soil from sites at the Sand Lake estuary. Six sites were determined, three in female-majority areas and three in male-majority areas; these sites were a random subset of the sites used in the mycorrhizal fungi collections in 2008 (described before). Soil was collected at least 1 m apart at eight locations at each of the six selected sites. Soil was collected from the top 10 cm, the depth at which *D. spicata* roots are most common (Sargeant et al., 2008). Samples were sent to the Cornell Nutrient Analysis Laboratory at Cornell University, Ithaca, New York, where they were analyzed for pH as well as suite of available nutrients: $NO_3$, K, P, Mg, CA, Fe, Al, Mn, Zn.

***Greenhouse experiment***—To determine whether male *D. spicata* plants growing in a common environment can interact with mycorrhizal fungi differentially, we grew plants vegetatively in the greenhouse with and without mycorrhizal fungi. We used plants that had been grown from seed (collected at the Pt. Reyes site in 2004), but had been growing vegetatively in the greenhouse at Portland State University for four years, never reaching sexual maturity. Plants remained prereproductive, and trays were rerandomized every two weeks for the first six months, every two months thereafter, and plants were randomized within trays every six months until they were used in the experiment. We determined the sex of 30 male and 30 female individuals from this collection of 1510 nonsexual *D. spicata* plants using a sex-specific, sequence-tagged site (STS) marker previously isolated (Eppley et al., 2009). Two separate 7 cm sections of rhizome were removed from each of the 60 individuals. All shoots, roots, and root hairs were removed from these rhizomes. On 1 February 2007, each rhizome was placed in a separate Cone-tainer (Ray Leach Cone-Tainers Nursery, Canby, Oregon, USA), with 55 ml of an autoclaved mixture of 1 part Black Gold "All Organic" potting soil (Sun Grow Horticulture, Vancouver, Canada) to 3 parts propagation sand. Soil mixture from the start of the experiment was sent to the Cornell Nutrient Analysis Laboratory, at Cornell University, Ithaca, NY, for analysis; initial soil fertility was low ($0.0 \pm 0.0$ ppm available $N0_3$, $3.1 \pm 0.2$ ppm available P, $34.0 \pm 0.9$ ppm available K, $52.4 \pm 0.92$ ppm available Mg, $343.8 \pm 5.5$ ppm available CA, $0.9 \pm 0.2$ ppm available Fe, $5.7 \pm 0.6$ ppm available Al, $13.8 \pm 0.2$ ppm available Mn, and $1.0 \pm 0.0$ ppm available Zn). Rhizomes were placed into one of two treatments: with or without mycorrhizal fungi. Mycorrhizal fungi for inoculant were obtained from the *Distichlis spicata* population at Sand Lake. Inoculant for each individual plant treated was produced by mixing 1 g of air-dried, ground soil with 5 mL of water. We placed 30 Cone-tainers per tray with Cone-tainers grouped by treatment to avoid mycorrhizal fungi contamination, and randomized trays in the greenhouse. Plants were watered by hand on a weekly basis. After 96 d, 10 g of root material was collected from each of the 29 surviving plants for AMF colonization analysis (detailed later). Plants were dried for 48 h at 60°C, and mass was determined. Biomass and plant nutrient status between sexes with and without AMF colonization could not be further quantified because initial results showed a sex bias in the fraction of plants colonized by AMF, with only three males colonized (see later). We thus only analyzed the fraction of plants colonized by AMF in males and females. Further experiments, which we are undertaking, will be needed to explore sex-specific effects of AMF colonization on growth and nutrient status; previous studies in *D. spicata* have shown positive effects of mycorrhizal fungi on growth, depending on environmental conditions (Allen and Cunningham, 1983).

***AMF colonization***—Roots were taken from storage at 4°C and washed with tap water. All collected roots within a sample were stained with Trypan blue, following Koske and Gemma (1989). After staining, fifty 1-cm root segments from each plant were randomly chosen and mounted on slides. AMF colonization was quantified using the magnified intersection method of McGonigle et al. (1990) and examined at 400×. Following Treseder and Vitousek (2001), we report our results as the percentage of examined root length with mycorrhizal structures.

***Data analysis***—To determine whether the sex ratio around male and female focal plants differed at the Sand Lake estuary and thus to assess within-population

sex ratio variation, we used a one-way ANOVA (SAS Institute, 2007). For field-collected plants in 2007, to determine the effect of plant sex (fixed), population (random), and the interaction between these two factors on the percentage of examined roots of adults that were colonized by AMF, we used a mixed-model ANOVA (SAS Institute, 2007). To ensure that the residuals were normally distributed and variances were homogeneous, the data were arcsine-square root transformed. For field-collected plants in 2008, we used a nested, mixed-model ANOVA to test how site (random, nested in site sex), site sex (fixed), season (fixed), and the interaction between site sex and season affected the percentage of examined roots that were colonized by AMF in *D. spicata* plants. We tested this same model using only colonization of arbuscules, rather than all mycorrhizal colonization (hyphae, arbuscules, and vesicles) because arbuscules have been found to have phenologies that do not necessarily match that of general colonization levels in some systems (Mullen and Schmidt, 1993). However, because these results were quantitatively and qualitatively similar to those for total AMF colonization, they were not reported.

For soil nutrient analyses, we used nested, mixed-model ANOVAs to determine whether each soil nutrient was affected by site (random, nested in site majority-sex) and site majority-sex, followed by a Bonferoni correction for multiple analyses. For the soil spore analysis, we used a one-way ANOVA to determine whether the total number of mycorrhizal spores differed between soil collected from majority-male and majority-female sites (SAS Institute, 2007). We analyzed spores found in the 38–250 μm fraction separately from the 250–500 μm fraction and found no significantly differences in spore numbers between the majority sex sites for either size class, so we combined the data for the final analysis to simplify the results presented.

For the greenhouse experiment, we used a logit model following Christensen (1990) to determine the effect of plant sex and tray on whether a plant was colonized by AMF (SAS Institute, 2007). The vast majority of male plants were not colonized by fungi, and thus we had too small a sample size to analyze differences in AMF colonization in examined roots between the sexes. Females and males did not differ significantly in any measure of mass, and the number of colonized males was insufficient to compare the effect of AMF colonization in examined roots on fitness measures such as mass. As expected, our control treatment without mycorrhizal fungi innoculant had a low level of mycorrhizal contamination (one root was found to have mycorrhizal fungi in the noninoculated treatment).

## RESULTS

***Population sex ratio at Sand Lake***—The Sand Lake population showed within-population sex ratio bias: *D. spicata* neighborhoods around female plants had a mean sex ratio of 0.32 ± 0.05 (SE), while *D. spicata* neighborhoods around male plants had a mean sex ratio of 0.73 ± 0.04 (SE). An ANOVA suggests that sex ratios around female and male focal plants were significantly different ($N = 60$; $F = 46.20$; df = 1; $P < 0.0001$; Fig. 1). Previous work in *D. spicata* populations on the west coast of the United States demonstrates that these populations are genetically diverse at small spatial scales (Eppley et al., 1998), suggesting that this pattern of within-population sex ratio bias is not created simply by clonal spread by a few genets but represents spatial segregation of hundreds of male and female genets.

***AMF colonization levels in the field 2007***—Sex-specific AMF colonization in examined roots was observed in adult *D. spicata* plants in the two field populations. The examined roots of adult *D. spicata* female plants from the field had significantly higher colonization by AMF than the examined roots of adult *D. spicata* male plants from the field (Table 1, Fig. 2). There was no significant affect of population or interaction between population and sex.

***AMF colonization levels in the field 2008***—Examined *D. spicata* roots from majority-female sites had significantly higher AMF colonization than examined *D. spicata* roots from majority-male sites (Table 2), reflecting a similar pattern of sex-specific colonization to that we found in populations in 2007. However, we also found that AMF colonization has a seasonal component in this salt marsh system. Colonization was significantly higher when plants were not flowering than when they had set seed (Fig. 3A). There was no significant interaction between the majority sex of a site and season, suggesting that majority-female sites showed a similar pattern of higher AMF colonization as majority-male sites across seasons (Fig. 3B, C).

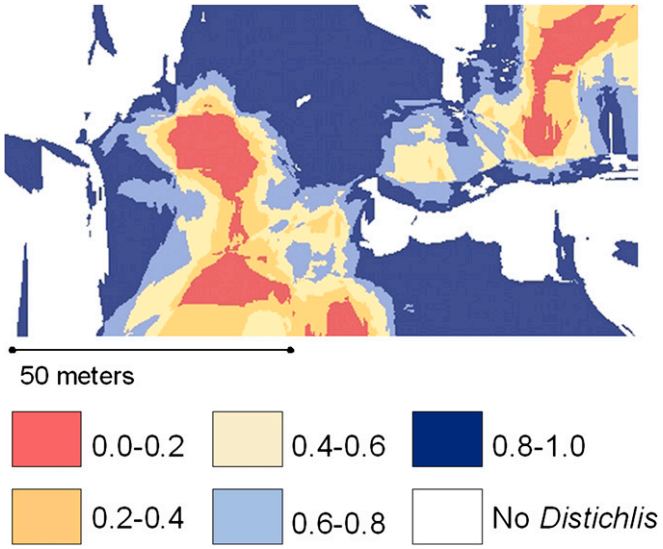


Fig. 1. Map of a subsection of the Sand Lake, Oregon site showing skewed within-population sex ratios. Orange areas represent areas with high concentrations of female *Distichlis spicata* plants (sex ratios = 0.0–0.2) and are frequent. Dark blue areas represent areas with high concentrations of male *D. spicata* plants (0.8–1.0) and dominate most of this marsh. Areas with even sex ratios (yellow; sex ratio = 0.4–0.6) are fairly rare. The map was generated using the program ArcGIS 9.2 (ESRI, Redlands, California, USA).

***Soil fertility and spore data***—At the Sand Lake field site, phosphorus levels were significantly higher in majority-female sites compared with majority-male sites in October during seed set (Appendix S1; see Supplemental Data with the online version of this article; Fig. 3D). Other nutrients tested, however, did not differ significantly between majority sex sites, although the majority of nutrients showed significant effect of subsite within majority-sex patches ($N0_3$, Mg, CA, Al, Zn). A significant difference for phosphorus between majority-female and majority-male sites was also found for an earlier data set from a *D. spicata* marsh at Tomales Bay, California (Eppley, 2000), although other micronutrients such as Mg, Ca, and Zn were higher in majority-female vs. -male sites in the earlier study.

TABLE 1. We used a mixed-model ANOVA to test how plant sex (fixed) and population (random) affect colonization by AMF in *D. spicata* plants collected from the field in 2007. Significant *P*-values are in boldface.

| Source of variation | df | *F* | *P* |
|---|---|---|---|
| Plant sex | 1 | 6.36 | **0.02** |
| Population | 1 | 0.72 | 0.40 |
| Plant sex × population | 1 | 1.08 | 0.31 |
| Error | 35 | | |

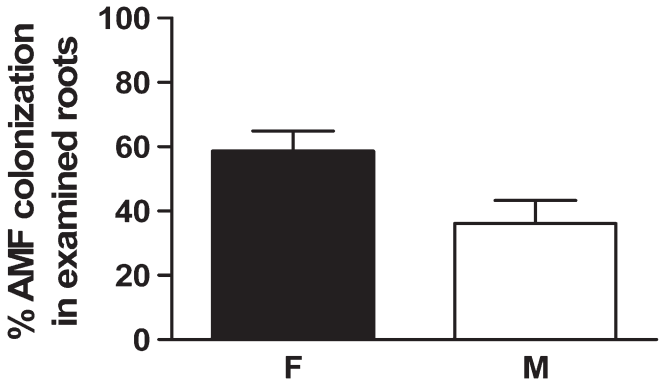


Fig. 2. Percentage of analyzed root colonized by AMF (+ SE) in female and male *Distichlis spicata* plants, 2007. Roots were collected when plants were flowering from Pt. Reyes, California and Sand Lake, Oregon. Colonization rates between populations did not differ ($N = 39$).

However, this previous study did not use the more conservative subsite sampling protocol used here, which may be why these additional nutrients were found to vary. Nitrogen was not found to be significantly different between majority-female and majority-male sites in either study. In this study we analyzed only $NO_3$ levels, but in the previous study, we analyzed both $NO_3$ and $NH_4$ levels and found that neither differed between majority-male and majority-female sites.

No significant differences were found in the number of mycorrhizal spores found in soil between majority-female and majority-male sites ($N = 13$; $F = 0.79$; $P = 0.39$). Soil from female-majority sites at Sand Lake, Oregon had a mean of 605.09 spores/g ± 284.37 (SE), while soil from majority-male sites had a mean 1057.95 spores/g ± 403.9 (SE). *Distichlis spicata* is the dominant plant at these sites, but other plant species at the site have the potential for mycorrhizal infection. Therefore, majority-female and majority-male sites may differ in the number of mycorrhizal fungi spores in soil that infect *D. spicata* specifically, despite these results.

***AMF colonization in the greenhouse***—In the greenhouse, female plants were significantly more likely to be colonized by AMF than were male plants (Fig. 4; $\chi^2 = 4.19$; df = 1; $P = 0.04$). Tray was not a significant factor ($\chi^2 = 1.68$; df = 1; $P = 0.20$). Because only three males were colonized by AMF, we did not statistically analyze whether levels of AMF colonization in examined roots differed between females and males in this experiment, although the percentage of examined roots that were colonized in females appeared to be higher than in males (24.67 ± 5.49 [SE] and 12.00 ± 5.29 [SE], respectively).

TABLE 2. Results of nested, mixed-model ANOVA to test how site (random, nested in site sex), site sex (fixed), and season (fixed) affected colonization by AMF in *D. spicata* plants collected from the field in 2008. Significant *P*-values are in boldface.

| Source of variation | df | *F* | *P* |
|---|---|---|---|
| Site (site sex) | 6 | 1.68 | 0.13 |
| Site sex | 1 | 33.95 | **0.0011** |
| Season | 1 | 15.14 | **0.0002** |
| Site sex × season | 1 | 0.11 | 0.74 |
| Error | 118 | | |

## DISCUSSION

***Sex-specific colonization***—Our data from populations in California and Oregon over two years demonstrate that there are differences in AMF colonization between the examined roots of male and female *D. spicata* plants. Populations showed roughly similar patterns of sex-specific colonization of examined roots (females had AMF colonization that was 1.62–2.58 times higher than males; Figs. 2 and 3), sex-specific differences in colonization were similar from summer to fall in the field, and the differential colonization occurred despite the fact that majority-female and majority-male sites had similar levels of mycorrhizal spores. Differences in the fraction of analyzed roots with mycorrhizal fungi could be due to either differences in the total number of mycorrhizal fungi in plants or differences in the total root length between the sexes. Previous work on *D. spicata* has found no growth rates differences between the sexes (Eppley, 2001), but no studies have looked specifically at belowground growth with AMF colonization, and we were unable to obtain sufficient AMF colonization in males in our greenhouse manipulation experiment to make the comparison. However, both mechanisms—differential AMF colonization and differential root growth between the sexes—have direct implications for intersexual competition in the *D. spicata* system, which has an extreme variation in the within-population sex ratio.

Our greenhouse experiment suggests that male and female *D. spicata* plants do differ in how they interact with mycorrhizal fungi. In the greenhouse, female plants were more likely to be colonized at all than were male plants (Fig. 4). Because male and female individuals were grown under analogous conditions and had access to similar levels of mycorrhizal fungi in the greenhouse, differences in AMF colonization between the sexes appear to be due to differences between how males and females interact with mycorrhizal fungi, even before reproductive maturity, rather than with growing conditions.

To the best of our knowledge, this study is the first to show that male and female plants are colonized differentially by AMF, although a previous study demonstrated that male and female plants benefited differentially from mycorrhizal infection (Varga and Kytöviita, 2008). There are little data available on whether males and females differentially interact with their potential mutualistic partners in other species with dimorphic sexual systems. Rarely, sex-specific differences may occur in pollinator mutualisms (Hemborg and Bond, 2005), and a sex-specific mutualistic interaction has been found to occur in the hermit crab *Pagurus longicarpus* in which males interact more frequently than females with the shell epibiont *Hydractinia symbiolongicarpus* (Bach et al., 2006). In plant systems, much of the research on sex-specific differences has focused on how variation in response to environmental stress will influence population sex ratios and structure (Geber et al., 1999). To a lesser degree, researchers have investigated sex-specific antagonistic interactions within the community (Ågren et al., 1999). However, mutualistic relationships have the potential to play an equal role in shaping community and population structures, and species that show sex-specific effects in interactions with their environments and other community members might also be expected to interact differentially in positive relationships.

***Seasonal variation***—In our field data, we found that AMF colonization in *Distichlis spicata* varies seasonally (Fig. 3A). AMF colonization is high early in the growing season, when

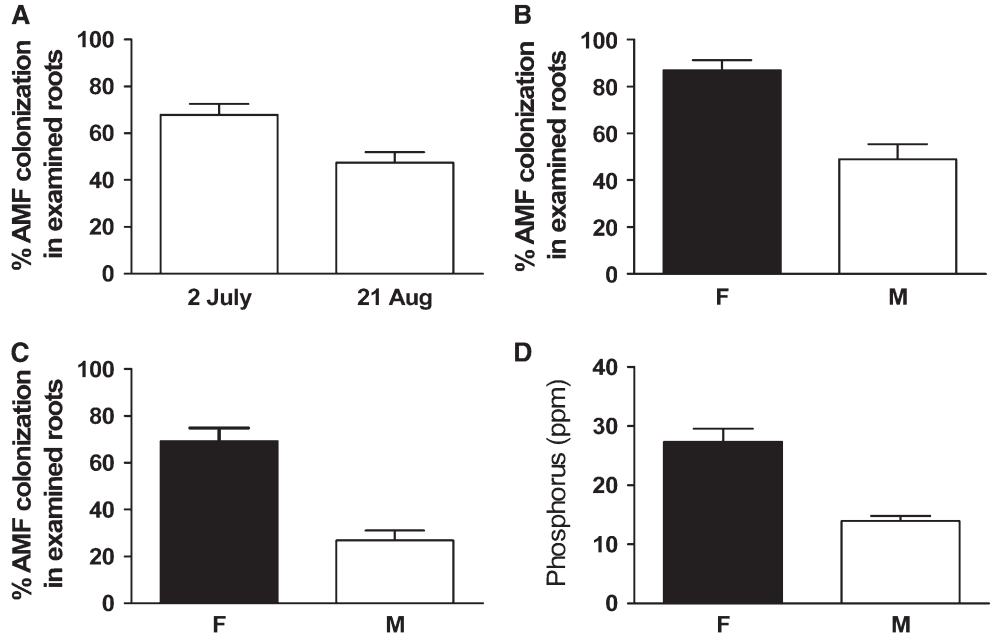


Fig. 3. Percentage of analyzed root colonized by AMF (+ SE) in *Distichlis spicata* plants at Sand Lake, Oregon in (A) all sites for the 2 July 2008 collections when plants were green but not reproductive vs. the 21 August 2008 collections when females were setting seed ($N = 128$); (B) majority-female vs. majority-male sites for 2 July ($N = 128$); and (C) majority-female vs. majority-male sites for 21 August ($N = 128$). (D) Soil phosphorus levels (ppm) at majority-female and majority-male sites (+ SE) for 21 August 2008 ($N = 48$).

plants resprout vegetatively after the long winter, and decreases as the season progresses. As with the sex-specific differences, this result could be due to differences in total AMF colonization at the plant level or to differences in root length between the seasons. The pattern we found, however, is similar to that documented in temperate freshwater wetlands (Bohrer et al., 2004), where maximum plant growth occurs in spring/early summer as does maximum AMF colonization, while flowering times for most species is later, and AMF colonization is much reduced by the end of the summer. Other studies, in salt marsh systems also suggest that AMF colonization is highest when vegetative growth rates are high (van Duin et al., 1990; but see Stenlund and Charvat, 1994; Carvalho et al., 2001), which is most likely in early summer in the *D. spicata* marshes in Oregon.

Perhaps surprisingly, we found that the sex-specific AMF colonization in examined roots of *D. spicata* was constant from early summer to fall (Fig. 3B, C), despite the fact that females had set seed in the second time period. Females are expected to have a higher allocation to sexual reproduction than males by the fall and thus might have been expected to have higher nutrient needs than males, which mycorrhizal fungi, acting as mutualists, might supply. The constant nature of the sex-specific interaction in *D. spicata* through the summer and early fall suggests that differential sex allocation is not a likely driver of the sex-specific AMF colonization rates we measured. Also, the plants used in our common-garden experiment never reached reproductive maturity and yet had a pattern of sex-specific AMF colonization (male plants were significantly less likely to be colonized than were female plants), adding weight to the suggestion that patterns of sexual allocation are not driving sex-specific AMF colonization variation in this system.

***Potential ecological and evolutionary consequences***—Intersexual competition in *D. spicata* is likely influenced by the sex-specific symbiosis with mycorrhizal fungi because differential mycorrhizal colonization in males and females may lead the sexes to have differential access to resources. Mycorrhizal relationships have been proposed to change competitive plant interactions by either (1) increasing competition because individuals have greater access to scarce resources (Facelli et al., 1999) or (2) decreasing competition because limited resources are shared via a hyphal network (Shumway and Koide, 1995). Empirical results suggest that AMF colonizations are likely to exacerbate competitive interactions, rather than reduce them (Allsopp and Stock, 1992; Shumway and Koide, 1995; Facelli et al., 1999). Thus, we expect that the sex-specific interaction with mycorrhizal fungi and *D. spicata* magnifies differential competitive effects between males and females, intensifying intersexual competition. Because mycorrhizal interactions also

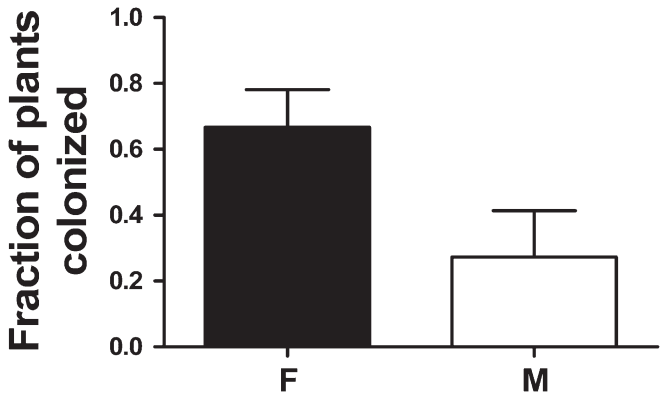


Fig. 4. Results from a greenhouse experiment with *Distichlis spicata* showing the fraction of female and male plants colonized by AMF (+ SE; $N = 29$).

increase size inequalities among plants within species (Moora and Zobel, 1998), any advantage that AMF colonization may confer to one sex is likely to be increased in intersexual competition but not in intrasexual competition.

Intersexual competition has been implicated as one probable mechanism responsible for the spatial segregation of the sexes in *D. spicata* populations where higher nutrient sites have nearly 100% female majorities and lower nutrient sites have nearly 100% male majorities (Eppley et al., 1998; Eppley, 2000, 2006). Determining the mechanisms responsible for spatial segregation of the sexes in plants is important for understanding dioecious plant population biology, as the pattern has been documented in over 25 plant species from 18 families (Bierzychudek and Eckhart, 1988; Iglesias and Bell, 1989; Korpelainen, 1991; Shea et al., 1993; Lokker et al., 1994; Eppley et al., 1998; Bertiller et al., 2002; Stark et al., 2005; Dudley, 2006). One potential mechanism responsible for spatial segregation of the sexes in *D. spicata* is intersexual competition, such that female plants may outcompete male plants for high phosphorus sites in the marsh, because phosphorus is the one nutrient we found to differ between female- and male-majority sites. Preferential interactions with mycorrhizal fungi for female plants over male plants may expedite this outcome. While typically mycorrhizal fungi react negatively to high rates of nutrients (Treseder, 2004), our results from the field site at Salt Lake, Oregon and those taken previously in California (Eppley, 2005) suggest that even majority-female sites in the *D. spicata* salt marsh have low levels of phosphorus and are nutrient limited. Whether majority-female sites are phosphorus limited and mycorrhizal fungi play a role in intersexual competition remains to be seen. Why males do not benefit equally from a similar high level of mycorrhizal interaction and establishment in relatively higher-nutrient sites also remains unclear. Females may have higher resource needs than males due to the presumably higher costs they pay for sexual reproduction (due to the high costs of maturing fruit), which may mean that they benefit more from high rates of AMF colonization than do males. Further manipulative experiments, which we are pursuing, are needed to determine how the sex-specific relationship between mycorrhizal fungi and *D. spicata* influences intersexual competition above and belowground and the sex ratios in this system. Investigations are also needed into how the interaction between sex allocation and phosphorus influences AMF colonization in *D. spicata*.

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