Mycorrhiza (2008) 18:257–261
DOI 10.1007/s00572-008-0178-1

SHORT NOTE

Compatible host/mycorrhizal fungus combinations for micropropagated sea oats: II. Field evaluation

Abid Al Agely · David M. Sylvia

Received: 26 February 2008 / Accepted: 16 May 2008 / Published online: 7 June 2008

Abstract Sea oats (*Uniola paniculata* L.) are the dominant plant in the pioneer coastal dunes of Florida and are widely used for dune restoration. DNA analysis has revealed significant ecotypic variation among Atlantic and Gulf coast populations of sea oats, but little is known about the diversity of the arbuscular mycorrhizal (AM) communities present in the dune systems. In a prior greenhouse study, we evaluated the functional diversity that exists among the AM fungal communities from divergent Florida dunes and selected effective host/AM fungus combinations for further study. The objective of this study was to evaluate the effect of these compatible combinations on the growth of sea oats planted at Anastasia State Recreation Area (AN) on the Atlantic coast and St. George Island State Park (SG) on the Gulf coast. Micropropagated sea oats from each site were inoculated with AM fungal communities also from AN and SG or a microbial filtrate control. The complete factorial of treatment combinations were grown in the greenhouse for 8 weeks and outplanted to the AN and SG field sites. After 1 year, root colonization was evaluated, and after 2 years, root colonization, shoot and root dry masses, and shoot- and root-P contents were determined. Overall, sea oats planted at AN had greater percent root colonization, shoot dry mass, and shoot-P content than those planted at SG. At AN, the local sea oat ecotype responded more to the fungal community from the same site relative to shoot dry mass and shoot-P content. At SG, the local fungal community produced larger plants with greater P content regardless of the origin of the host. We conclude that sea oat productivity is responsive to AM fungal ecotype as well as host ecotype, and fungal origin should therefore be taken into account when planning sea oat plantings on coastal dunes.

Keywords Dune ecology · Ecotype · Effectiveness · Field performance · Florida

A. Al Agely (✉)
Soil and Water Science Department, University of Florida,
2196 McCarty Hall A, P.O. Box 110290,
Gainesville, FL 32611, USA
e-mail: aaag@ufl.edu

D. M. Sylvia
Crop and Soil Sciences Department,
Pennsylvania State University,
116 ASI Bldg,
University Park, PA 16802, USA

Introduction

Coastal plants provide an effective defense against the erosive forces of wind and waves (Woodhouse 1982), and revegetation is the method of choice for the restoration of sand dunes (Dean 1983). In the southeastern USA, sea oats (*Uniola paniculata* L.) are the dominant plant in the pioneer zone of coastal sand dunes and are widely used in dune restoration (Woodhouse et al. 1968).

Plant and microbial variation likely have roles in the establishment and growth of sea oats on coastal dunes. DNA analysis has revealed that sea oats on Florida coasts are genetically different (Ranamukhaarachchi et al. 2000). Ecotypic variation was reported both within and between sea oat populations of the same and different coasts. Sea oats on the Gulf coast displayed the greatest genetic variations, while those on the Atlantic coast had lower genetic differentiation. Less is known about the diverse communities of arbuscular mycorrhizal (AM) fungi present in these dune systems (Sylvia 1986). However, genetic variation among AM fungi has been reported by others

(Boerner 1990; Boyetchko and Tewari 1995; Monzon and Azcon 1996; Stahl et al. 1990; Stahl and Smith 1984), supporting the hypothesis that ecotypic variation among AM fungi may have an impact on the establishment and growth of coastal plants.

Scientists at the University of Florida (Sylvia et al. 2003; Valero-Aracama et al. 2007) are developing protocols for coastal dune restoration that use micropropagated and AM fungus inoculated sea oats. Coastal sand dunes are generally characterized by low nutrient availability and natural disturbances and are likely candidates to benefit from mycorrhizal applications (O'Keefe and Sylvia 1991). Previous studies documented growth response of sea oats to AM inoculation in commercial nurseries and coastal sand dunes (Sylvia 1989; Sylvia et al. 1993). Furthermore, in a prior greenhouse study, we evaluated the functional diversity that exists among communities of AM fungi present in divergent Florida dunes and selected effective sea oat/AM fungus combinations for field testing (Sylvia et al. 2003). The objective of the present study was to evaluate the response of these compatible sea oat/AM fungal ecotype combinations on the establishment and growth of sea oats planted on selected Atlantic and Gulf coast sand dunes.

Materials and methods

The Atlantic coast site was at Anastasia State Recreation Area (AN) located in northeastern Florida (29°53′15″N, 081°17′23″W), and the Gulf coast site was at St. George Island State Park (SG) located in northwestern Florida (29° 39′20″N, 084°52′53″W). The pH and water-extractable phosphorus (P, μg ml^{-1}) concentrations, respectively, at each location were: AN, 8.3 and 25.9 and SG, 7.6 and 5.0.

The sea oat/AM fungus treatment combinations were as follows: Sea oat ecotypes (AN-07-4-1 from the Atlantic coast and SG16-1-2 from the Gulf coast) were inoculated with two AM fungal communities (AN9 from the Atlantic coast and SG1 from the Gulf coast) and a control treatment. Micropropagated sea oats (Philman and Kane 1994) were placed in a greenhouse on 10 July 2002 for 8 weeks at 34° C and 22°C maximum and minimum mean temperatures, respectively, and mean maximum photosynthetic photon flux density of 1,240 μmol m^{-2} s^{-1}. The growth medium was acid-washed builder sand mixed 1:1 with coarse vermiculite, moistened, and pasteurized twice at 85°C for 8 h with 48 h between heating. The medium was then placed into containers (4-cm diameter by 18-cm depth) and mixed with 10 g (dry mass basis) of soil and colonized

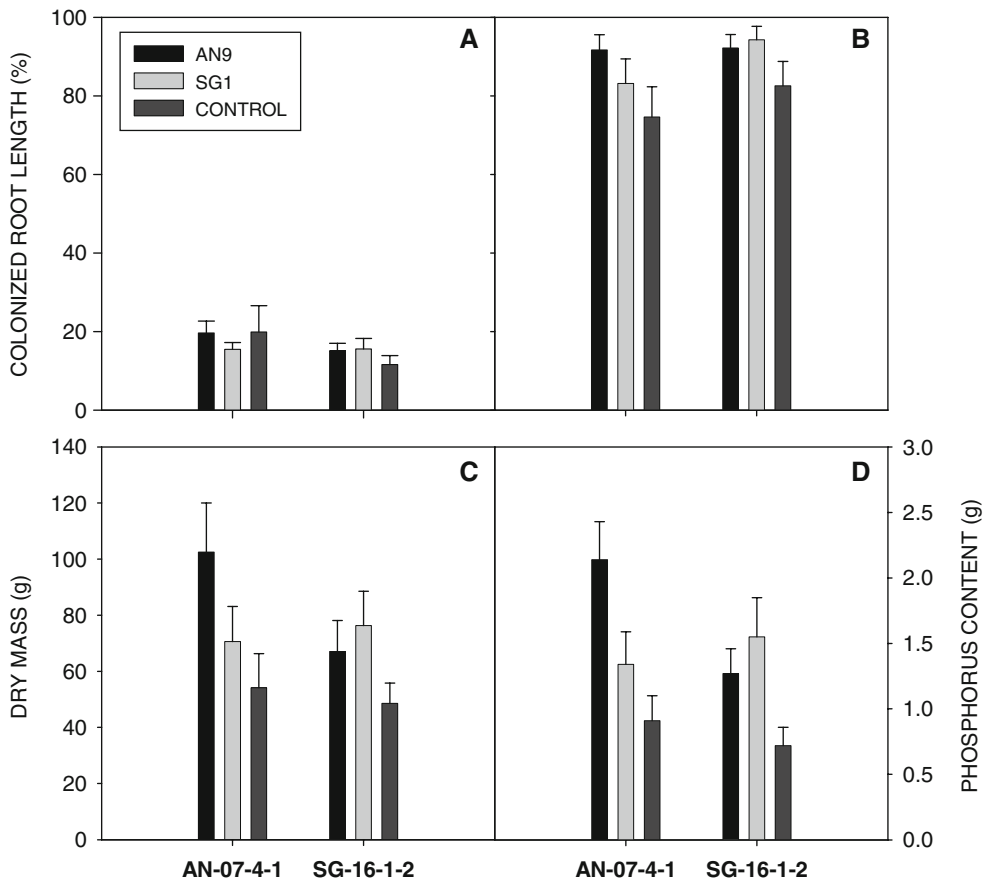

Fig. 1 Effect of sea oat ecotype and AM fungal community on root colonization after one (**a**) and two (**b**) growing seasons, shoot dry mass (**c**), and shoot-P content (**d**) at harvest of sea oats planted at an Atlantic coast site. *Bars* represent the means of 12 replicates ± SEM

roots from sweet corn (*Zea mays* L.) pot cultures of the respective AM fungal community. The control treatment received autoclaved pot culture inocula from both fungal communities amended with live microbial filtrate (<20-μm pore size) to reintroduce a portion of the microflora other than AM fungi (Ames et al. 1987). Before the seedlings were outplanted, ten replicates per treatment combination were selected randomly to assess mycorrhizal colonization and shoot dry mass before outplanting.

Sea oat seedlings were transplanted at the AN site on 5 October 2002 and at the SG site on 12 October 2002. Five blocks were initially established at each site, with at least 50 m between blocks. The six treatment combinations (two hosts: AN-07-4-1 and SG16-1-2; three AM inocula: AN9, SG1, and control) were placed randomly within blocks, each consisting of a plot of 16 plants (4×4 rows) on 50-cm centers with 10 m between plots. The planting holes were 10 cm deep, and plants were fertilized with a time-release fertilizer (Osmocote®, 13N-4P-5K, Grace Sierra Horticulture Products, Milpitas, CA, USA) at a rate of 1.24 g per plant.

After 1 year (19 and 26 October 2003, respectively, for AN and SG sites), cores (542 cm^3 each) were taken to a depth of 30 cm adjacent to four randomly selected and marked sea oat plants from each plot. After 2 years (17 and 24 October 2004, respectively, for AN and SG sites), the same four plants from each plot were excavated by removing approximately 8 l of roots and surrounding soil. The samples, which included growth from rhizomes and the original plants, were sealed in labeled plastic bags and placed in an ice chest for transport back to the laboratory.

The roots were removed from samples by wet sieving, and fresh weights were determined. Roots were then cut into 2.5-cm lengths, and subsamples (0.5 g) were processed for assessment of AM colonization (Sylvia 1994). Shoots and the remainder of the roots were dried at 68°C, weighed, ground to pass a 20-mesh screen, dry-ashed, and P content determined (Murphy and Riley 1962).

Data were statistically analyzed using PROC MIXED and PROC GLM (SAS Institute 2003) to test for main effects and their interactions on all response variables. Population normality was tested for each variable before using parametric statistics for comparison testing. The field sites were unfortunately damaged during the active hurricane seasons of 2003 and 2004. Two blocks were destroyed at SG, while the damage at AN was scattered among blocks. Samples were combined from three less-damaged AN blocks to permit balanced data analyses.

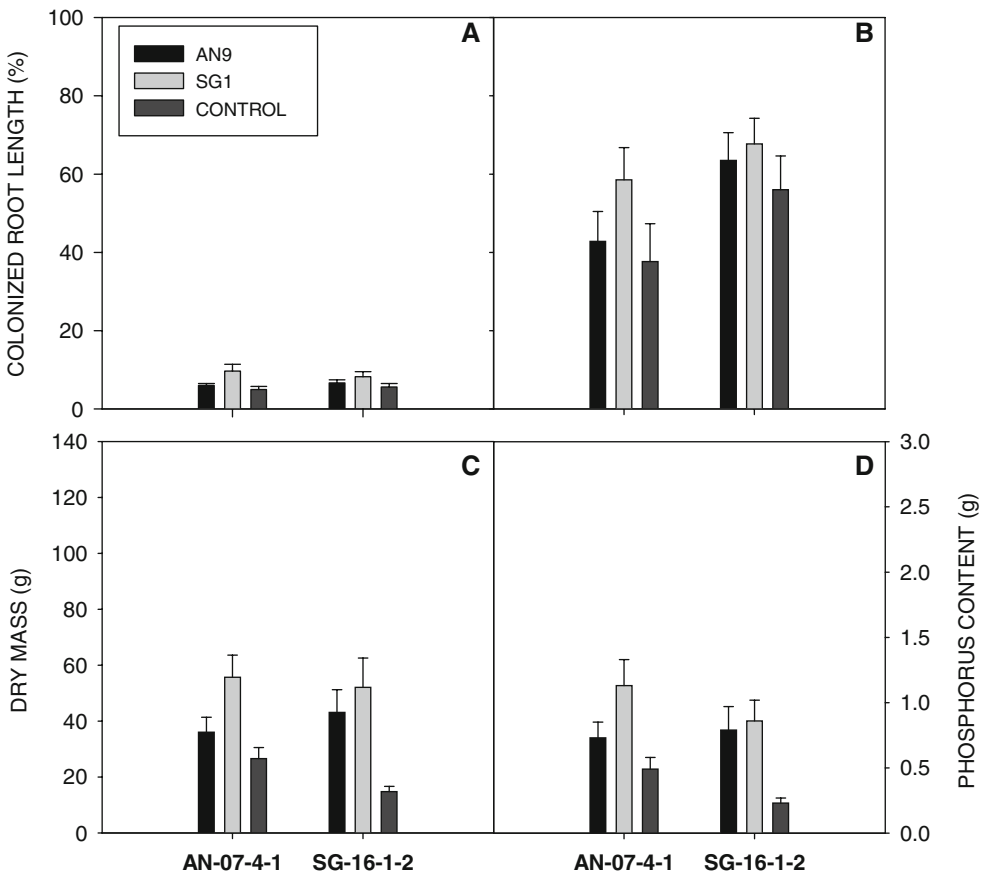

Fig. 2 Effect of sea oat ecotype and AM fungal community on root colonization after one (**a**) and two (**b**) growing seasons, shoot dry mass (**c**), and shoot-P content (**d**) at harvest for sea oats planted at Gulf coast site. *Bars* represent the means of 12 replicates ± SEM

Results and discussion

Plant analysis just before outplanting indicated that inoculation with the Atlantic coast mycorrhizal community resulted in greater root colonization (33%±5.4) and shoot dry mass (640 mg±97.7) than with the Gulf coast mycorrhizal community (13%±2.7 and 370 mg±67.5, respectively). These results are in contrast to our prior greenhouse study (Sylvia et al. 2003) where SG1 resulted in superior plant growth across plant ecotypes from all locations. Nonetheless, all AM fungal inoculated plants were colonized at outplanting, and controls were not colonized.

At the AN site on the Atlantic coast, shoot dry mass (P=0.0498) and shoot-P content (P=0.0013) varied significantly with treatment combination (Fig. 1). No significant differences were found between treatments relative to AM fungal colonization during the first and second year nor for root dry mass or root-P content (data not presented), probably due to the establishment of mycorrhizal associations native to the habitat. The local sea oat ecotype, AN-07-4-1, responded more to the fungal ecotype, AN9, from the same site relative to shoot dry mass and shoot-P content than did the sea oat ecotype from SG. In contrast, the SG sea oat ecotype tended to have greater shoot mass and P-content when inoculated with the fungal community from the same site, but this was less than when the plant, fungus, and site were all the same.

At the SG site on the Golf coast, shoot dry mass (P=0.0005) and shoot-P content (P=0.001) were also significantly affected by the treatment combinations (Fig. 2). At this site, the SG fungal community produced larger plants with greater P content regardless of the origin of the host plant.

This study confirms that host productivity is responsive to AM fungal ecotype and supports previous studies in the same region that demonstrated growth responses to mycorrhizal inoculation (Sylvia et al. 2003; Sylvia and Burks 1988). The evidence regarding the host ecotype was mixed, with a strong host impact at AN, but less so at SG. Other studies in different environments and with different hosts also suggest specificity between the plant host and the mycorrhizal fungi that may be ecologically important (Aldrich-Wolfe 2007; Jumpponen et al. 2004; Talukdar and Germida 1994; Zhu et al. 2000).

The results of this study could be used by federal, state, and local authorities to develop better protocols for the restoration of Atlantic and the Gulf coast dunes. For maintaining the stability of Florida coastal sand dune ecosystems, the ecotypic variation of AM fungi may be just as important as the ecotypic variation that occurs in the host plant. Resource managers should be cognizant of sea oat and AM fungal ecotypic compatibility with the planting site when planning dune restoration projects.

Acknowledgment Partial support for this project was provided by the Office of Sea Grant, National Oceanic and Atmospheric Administration, US Department of Commerce (grant no. NA76RG0120). We thank Andy Ogram, Ioannis Ipsilantis, and Gyummo Ely Na for their support and technical assistance.

References

Aldrich-Wolfe L (2007) Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. Ecology 88:559–566 DOI 10.1890/05-1177

Ames RN, Mihara KL, Bethlenfalvay GJ (1987) The establishment of microorganisms in vesicular–arbuscular mycorrhizal and control systems. Biol Fertil Soils 3:217–223 DOI 10.1007/BF00640633

Boerner REJ (1990) Role of mycorrhizal fungus origin in growth and nutrient uptake by geranium. Am J Bot 77:483–489 DOI 10.2307/2444382

Boyetchko SM, Tewari JP (1995) Susceptibility of barley cultivars to vesicular–arbuscular mycorrhizal fungi. Can J Plant Sci 75:269–275

Dean RG (1983) Principles of beach nourishment. In: Kormar PD, Moore JR (eds) CRC handbook of coastal processes and erosion. CRC, Boca Raton, FL, pp 217–231

Jumpponen A, Claridge AW, Trappe JM, Lebel T, Claridge DL (2004) Ecological relationships among hypogeous fungi and trees: inferences from association analysis integrated with habitat modeling. Mycologia 96:510–525 DOI 10.2307/3762171

Monzon A, Azcon R (1996) Relevance of mycorrhizal fungal origin and host plant genotype to inducing growth and nutrient uptake in *Medicago* species. Agric Ecosyst Environ 60:9–15 DOI 10.1016/S0167-8809(96)01066-3

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36 DOI 10.1016/S0003-2670(00)88444-5

O'Keefe DM, Sylvia DM (1991) Mechanisms of the vesicular–arbuscular mycorrhizal plant-growth response. In: Arora DK, Rai B, Mukerji KG, Knudsen GR (eds) Handbook of applied mycology. Marcel Dekker, New York, pp 35–53

Philman NL, Kane ME (1994) Micropropagation of *Uniola paniculata* L. (sea oats).. Hortscience 29:559

Ranamukhaarachchi DG, Kane ME, Guy CL, Li QB (2000) Modified AFLP technique for rapid genetic characterization in plants. BioTechniques 29:858–866

SAS Institute (2003) The SAS system for windows. SAS Inst, Cary, NC

Stahl PD, Smith WK (1984) Effects of different geographic isolates of *Glomus* on the water relations of *Agropyron smithii*. Mycologia 76:261–267 DOI 10.2307/3793102

Stahl PD, Christensen M, Williams SE (1990) Population variation in the mycorrhizal fungus *Glomus mosseae*: uniform garden experiments. Mycol Res 94:1070–1076

Sylvia DM (1986) Spatial and temporal distribution of vesicular–arbuscular mycorrhizal fungi associated with *Uniola paniculata* L. in Florida foredunes. Mycologia 67:734–740

Sylvia DM (1989) Nursery inoculation of sea oats with vesicular–arbuscular mycorrhizal fungi and outplanting performance on Florida beaches. J Coastal Res 5:747–754

Sylvia DM (1994) Vesicular–arbuscular mycorrhizal (VAM) fungi. In: Weaver RW, Angle JS, Bottomley PJ, Bezdicek D, Smith S, Tabatabai A, Wollum AG (eds) Methods of soil analysis, Part 2. Microbiological and biochemical properties. Soil Science Society of America, Madison, WI, pp 351–378

Sylvia DM, Burks JN (1988) Selection of a vesicular–arbuscular mycorrhizal fungus for practical inoculation of *Uniola paniculata*. Mycologia 80:565–568 DOI 10.2307/3807859

Sylvia DM, Jarstfer AG, Vosátka M (1993) Comparisons of species and formulations of mycorrhizal fungi on diverse Florida beaches. Biol Fertil Soils 16:139–144 DOI 10.1007/BF00369416

Sylvia DM, Alagely AK, Kane ME, Philman NL (2003) Compatible host/mycorrhizal fungus combinations for micropropagated sea oats—I. Field sampling and greenhouse evaluations. Mycorrhiza 13:177–183 DOI 10.1007/s00572-003-0232-y

Talukdar NC, Germida JJ (1994) Growth and yield of lentil and wheat inoculated with 3 Glomus isolates from Saskatchewan soils. Mycorrhiza 5:145–152 DOI 10.1007/BF00202347

Valero-Aracama C, Wilson SB, Kane ME, Philman NL (2007) Influence of in vitro growth conditions on in vitro and ex vitro photosynthetic rates of easy- and difficult-to-acclimatize sea oats (*Uniola paniculata* L.) genotypes. In Vitro Cell Dev Biol, Plant 43:237–246 DOI 10.1007/s11627-006-9014-5

Woodhouse WW (1982) Coastal sand dunes of the U.S. In: Lewis RR (ed) Creation and restoration of coastal plant communities. CRC, Boca Raton, FL, pp 1–44

Woodhouse WW, Seneca ED, Cooper AW (1968) Use of sea oats for dune stabilization in the southeast. Shore Beach 36:15–21

Zhu YG, Laidlaw AS, Christie P, Hammond MER (2000) The specificity of arbuscular mycorrhizal fungi in perennial rye-grass-white clover pasture. Agric Ecosyst Environ 77:211–218 DOI 10.1016/S0167-8809(99)00087-0