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Arbuscular Mycorrhizae in Sand Dune Plants of the North Atlantic Coast of the U.S.: Field and Greenhouse Inoculation and Presence of Mycorrhizae in Planting Stock

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The ability of several sand dune-inhabiting plant species to successfully colonize dune sites appears to depend upon the presence in the soil of arbuscular mycorrhizal fungi (AMF) that form mutualistic associations with roots. Dune sites barren of vegetation lack these fungi whose large spores are not readily dispersed to the root zones. Despite the absence of these beneficial fungi from barren sites, however, plantings made in AMF-free dune soils eventually form the mycorrhizal association. Examination of planting stock of several species of plants that are used to vegetate barren sand dunes and dune flats of the eastern seaboard of the U.S.A. revealed that AMF were routinely present in the planting materials prior to outplanting. AMF occurred in planting stock of seven varieties of *Ammophila breviligulata*, and in *Prunus maritima*, *Rosa rugosa*, and *Spartina patens*, but were absent from *Myrica pensylvanica*. In a field planting in a previously barren deflation zone in the large parabolic dunes of Cape Cod National Seashore, Massachusetts, culms of *A. breviligulata* that were inoculated with native species of AMF produced more tillers and inflorescences than did non-inoculated plants, even though 78% of the latter had become mycorrhizal 47 weeks after planting. In greenhouse experiments, *P. maritima* was found to have an absolute requirement for AMF and *Solidago sempervirens* was not. The significance of the presence of AMF in planting stock for revegetation and restoration of previously unvegetated sites lacking in AMF is discussed.

Keywords: *Ammophila breviligulata*, arbuscular mycorrhizae, dune restoration, sand dune plants.

1. Introduction

Stabilization of large, mobile dune fields by plantings of vegetation has long been recognized as an effective means of slowing the inland movement of sand (e.g. Jagschitz and Bell, 1966; Hewett, 1970; Ranwell, 1972; Woodhouse, 1982). Large plantings have been made at Cape Cod, Massachusetts, for this purpose since at least 1825 (Strahler, 1966). The most recent major plantings at Cape Cod National Seashore (CCNS) have

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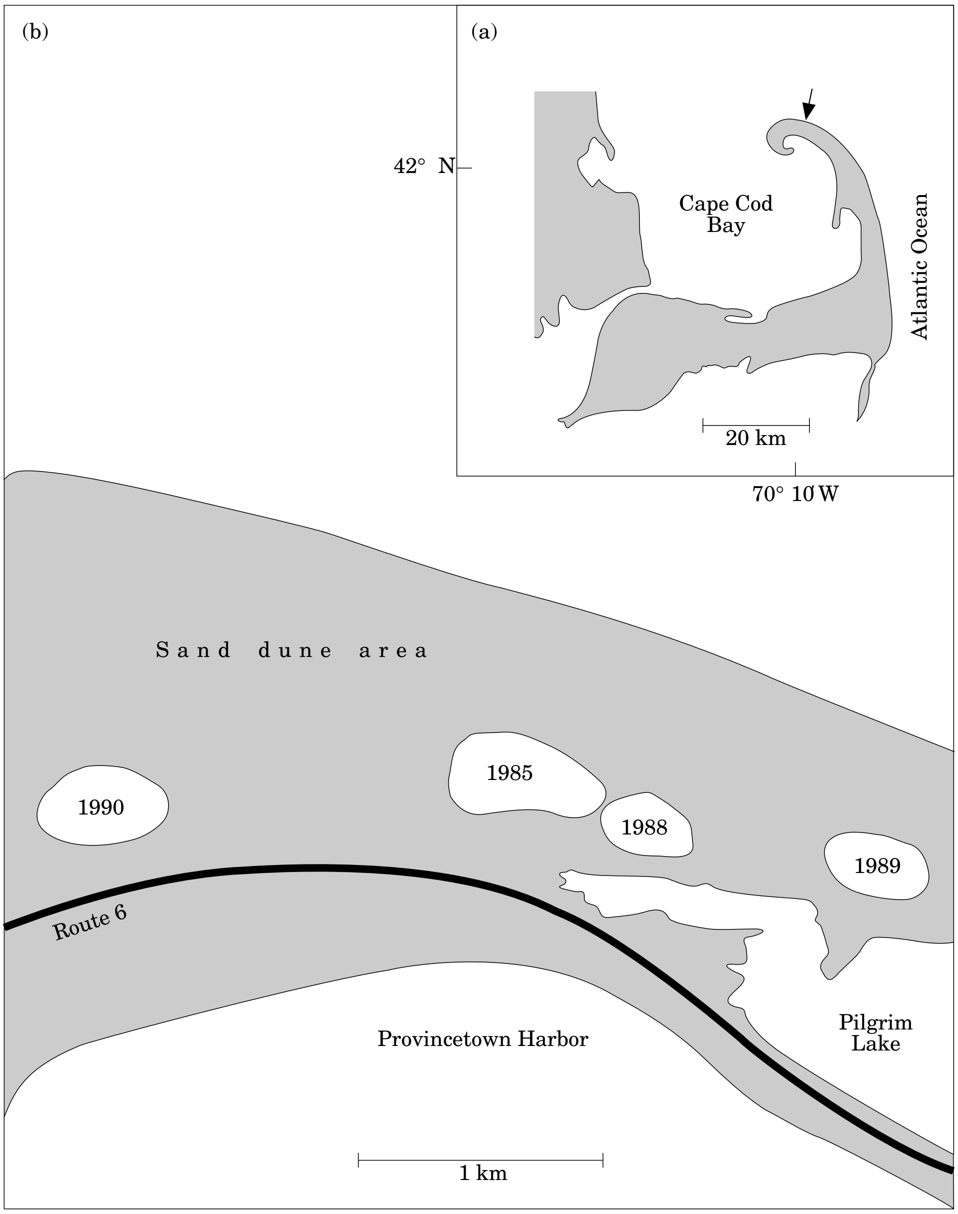

Figure 1. Location of the field study on Cape Cod, Massachusetts. A. General location. B. Area of the Province Lands dunes where plantings of *Ammophila breviligulata* have been made by the National Park Service in 1985, 1988, 1989 and 1990. Blowing sand is carried southward off the dunes onto Route 6 and into Pilgrim Lake and Provincetown Harbor. A section of the 1990 planting was inoculated with AMF.

been made in the Province Lands Dunes Area, a region of dune fields located near the north end of Cape Cod and measuring *ca.* 6 × 1·5 km (Figure 1). Since 1985, *ca.* 75 ha of American beachgrass (*Ammophila breviligulata* Fern.) have been planted (in 1985, 1988, 1989, and 1990) in a program administered by the National Park Service (NPS). Prior to these plantings, much of the existing vegetation in the dunes had been destroyed by grazing livestock and human use of the dunes for recreation. As a result, inundation of roads, buildings, and adjacent forests and the filling in of Provincetown Harbor and Pilgrim Lake by wind-blown sand are long-standing problems in the area.

The roots of plants that colonize lacustrine, inland and maritime sand dunes, including those of CCNS, are intimately associated with arbuscular mycorrhizal fungi [AMF, formerly called vesicular-arbuscular mycorrhizal fungi (VAMF)] (e.g. Nicolson, 1959, 1960; Nicolson and Johnston, 1979; Koske *et al.*, 1975; Koske, 1975, 1987, 1988; Jehne and Thompson, 1981; Koske and Halvorson, 1981, 1989; Giovannetti and Nicolson, 1983; Koske and Polson, 1984; Giovannetti, 1985; Sylvia, 1986; Puppi and Riess, 1987; Gemma and Koske, 1988, 1989, 1992; Rose, 1988; Dalpe, 1989; Read, 1989; Koske and Gemma, 1990, 1992, 1996, 1997; Abe *et al.*, 1994; Sturmer and Bellei, 1994; Abe and Katsuya, 1995; Al-Agely and Reeves, 1995). Based on the high incidence of mycorrhizae in the field and several growth experiments, it is thought that AMF are of vital importance to the establishment, growth and survival of the dominant plant species that colonize dunes (e.g. Koske and Polson, 1984; Puppi and Reiss, 1987; and see below). AMF appear to benefit plants by improving the uptake of phosphate and other nutrients from the soil, conferring increased drought and disease tolerance to the host plant, and contributing to soil structure and stability (e.g. Koske *et al.*, 1975; Sutton and Sheppard, 1976; Harley and Smith, 1983; Nelson, 1987; Newsham *et al.*, 1995). Because species of plants differ in their response to AMF in the soil, the presence or absence of AMF has been linked to the composition of the plant communities that develop in dune sites (Read, 1989; Koske and Gemma, 1990, 1992; Francis and Read, 1994, 1995).

While AMF have been found to be present in almost all natural sites (Harley and Smith, 1983), their propagules are rare or lacking from unvegetated dune sites (Sylvia and Will, 1988), including barren sites in the dunes of CCNS (Koske and Gemma, 1992, 1997). The fungi are obligate symbionts, able to grow and reproduce only when attached to living roots (Harley and Smith, 1983). The large spores of AMF are formed underground and appear not to be easily dispersed to new sites and move below the soil surface in barren sites. Further, if the vegetation of an area is destroyed, the population of viable propagules of AMF declines steadily (e.g. Miller, 1979; Reeves *et al.*, 1979) until it is too low to contribute to the successful establishment of plant species that can benefit from the symbiosis.

The most important species used for dune stabilization programs include American beachgrass (*Ammophila breviligulata* Fern.), European beachgrass (*A. arenaria* L.), and, in warmer climates, sea oats (*Uniola paniculata* L.). All three species have been studied for their response to inoculation with AMF (Nicolson and Johnston, 1979; Koske and Polson, 1984; Sylvia and Burks, 1988; Sylvia and Will, 1988; Gemma and Koske, 1989; Sylvia, 1989; Sylvia *et al.*, 1993), and, while results have been positive, large scale inoculation of new plantings in the field has not been incorporated into the planting protocol.

Plantings of these grasses are often made in areas that have lacked vegetation for several years or in sites composed of replenishment sand dredged from offshore. Yet, within a year after plantings made in these AM fungal-free dune soils, most plants have become mycorrhizal, apparently from fungi carried in the culms and root fragments of the planting stock (Sylvia and Will, 1988; Gemma and Koske, 1989).

Along the Mid- and North Atlantic coast of the U.S., the major species used for dune stabilization programs is *A. breviligulata*. For federally supported planting projects, plant materials typically originate from the USDA Soil Conservation Service, Plant

Materials Center (PMC) in Cape May, New Jersey. Stock from this center is distributed to commercial growers who multiply the plants and provide them to personnel involved in the actual planting of the grass in the dunes. Planting stock consists of single culms (tillers) 45–60 cm long, with most roots and dead leaves removed. Old roots up to 3 cm long often are present on the culms at the time of planting.

In addition to providing materials of *A. breviligulata*, the PMC offers a variety of other plant species for dune stabilization or restoration programs. For those species that are dependent upon arbuscular mycorrhizae, it would be necessary to ensure that AMF are present at planting. The absence of AMF from dune soils could limit the success of establishment if fungi were also absent from the planting stock.

The purpose of this study was to determining how AMF arrive in dune soils when plantings are made in barren sites. Four species of plants that are vegetatively propagated were tested for their ability to co-disperse AMF on their planting stock. In addition, we also assessed the effect of added AMF on the growth of three dune species to determine the importance of the symbiosis for plant establishment.

2. Materials and methods

2.1. MYCORRHIZAE IN DUNE-PLANTING STOCK

Culms of seven varieties of *A. breviligulata* with a few short roots (1–3 cm long) attached and rooted planting stock of the dune-colonizing species *Myrica pensylvanica* Loisel., *Prunus maritima* Marsh., *Rosa rugosa* Thunb., and *Spartina patens* (Ait.) Muhl. cv. Avalon were obtained from the PMC on 18 September 1991. Roots were removed from three individuals of each species or variety and were cleared, stained, and examined for the presence of arbuscular mycorrhizae (Koske and Gemma, 1989). Portions of each root systems were observed at 400 × magnification. Roots were considered to have functional arbuscular mycorrhizae only if arbuscules were observed (Gemma and Koske, 1990).

2.2. FIELD INOCULATION TRIALS

The field inoculation study was performed in the Province Lands Dunes region of CCNS (70° 8.5′ W, 42° 4.0′ N) near Provincetown, Massachusetts (Figure 1). The vegetation and ecological processes occurring in the Cape Cod dunes are similar to those in dunes along much of the North Atlantic seaboard of the U.S. (Godfrey, 1977; Godfrey *et al.*, 1979; Woodhouse, 1982). The geology of the area is described by Strahler (1966). The Province Lands Dunes comprise a variety of dune systems in various stages of succession. Large mobile dunes up to 32 m high are interspersed among smaller, more densely vegetated areas (Strahler, 1966). In the mobile dunes, the dominant plant species is *A. breviligulata*. Early invaders of *A. breviligulata*-dominated sites are *Polygonella articulata* (L.) Meisn. and *Solidago sempervirens* L. Later successional colonizers include the grass *Deschampsia flexuosa* (L.) Trin., the shrubs *Myrica pensylvanica* Loisel. and *Prunus maritima* Marsh., the trees *Pinus rigida* Mill. and *Quercus ilicifolia* Wang., and other species (Svenson and Pyle, 1979; Whatley, 1988).

In November 1990, the National Park Service (NPS) administered the planting of a 12 ha deflation zone with *A. breviligulata* cv. Cape (supplied by Church's Garden Center). Prior to planting, 15 soil samples (*ca.* one litre each) were collected for physical analysis and were examined for AMF by attempting to recover spores from the soil

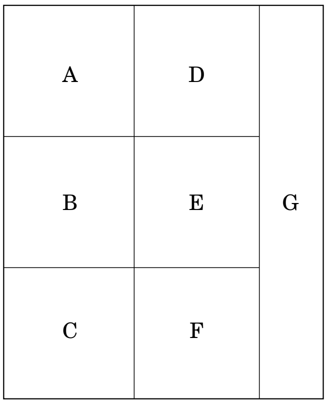

Figure 2. Inoculation plan for field planting of *Ammophila breviligulata* at Cape Cod. Plots A–F each contained 100 hills (10 rows of 10 hills each) and plot G contained 120 hills (30 rows with four hills in each row). Culms in plots C, D and E received inoculum of native AMF and plots B, F and G were not inoculated. Plot A was not used.

and by growing plants of *Zea mays* in soil samples for 8·5 weeks and examining the roots for the presence of mycorrhizae. No AMF were detected (Koske and Gemma, 1992, 1997).

One month before planting the pH of the quartz sand of the planting site was 6·0, with 1·5 ppm nitrate, 14·5 ppm phosphorus, 5·0 ppm potassium, and 0·12% organic matter. One week after planting and fertilization (see below) the soil characteristic were: pH 5·4, nitrate (2·1 ppm), phosphorus (24·5 ppm), and potassium (5·4 ppm) (Koske and Gemma, 1992).

On 23 November 1990, the seven plots (A–G) were marked off in a 11 × 14 m barren area near the center of the 12 ha planting site (Figure 2). Six of the plots (A–F) measured 4·6 × 4·6 m and consisted of 10 rows of plants spaced 46 cm apart, each row made up of 10 "hills", each hill spaced 46 cm apart. Total hill number for each of these plots was 100. Plot A was not used for the experiment described here, but for another study. There was one additional plot (G) measuring four hills by 30 rows (total of 120 hills).

There were two treatments that were randomly assigned to the plots. Plants in plots C, D, and E were inoculated with naturally occurring AMF contained in root zone soil collected from a native stand of *A. breviligulata* growing vigorously *ca.* 50 m from the plots. This soil/inoculum (50 ml) was placed at the bottom of an 20-cm-deep hole into which were placed three culms. The hole was closed by tamping the surface with the heel of the foot. Plots B, F, and G were the controls and received 50 ml of AM fungus-free dune sand in each planting hole in place of the inoculum sand. The sand used for the controls was collected three days prior to the planting from the same location from which the inoculum soil came. It was pasteurized (90°C × 2 hr) to kill propagules of AMF, cooled, and then watered with filtered washings from unsterilized sand from the same site to reestablish a similar microflora but lacking AMF. The filtered washings were prepared by stirring 600 ml dune soil in 1·2 l of water. The solution was allowed to stand for one hour, and the supernatant was filtered through Whatman no. 4 filter paper.

Spores of AMF present in the sand used for inoculum were isolated by wet-sieving/sucrose centrifugation (Walker *et al.*, 1982). Two 100 ml soil samples were analysed. Species present were *Gigaspora margarita* Becker and Hall (average 47 spores/100 ml), *Glomus clarum* Nicol. and Schenck (1/100 ml), and *Scutellospora calospora* Nicol. and Gerd., Walker and Sanders (0·5 spores/100 ml). Soil pH and nutrients were as follows: pH (6·0 in soil used for inoculum, 5·6 in soil used for control); nitrate (1·9 ppm in inoculum, 1·6 ppm in control), phosphorus (14·0 ppm in inoculum, 13·5 ppm in control), potassium (5 ppm in inoculum, 5 ppm in control) (Koske and Gemma, 1992).

After planting, the plots and adjacent commercial plantings were fertilized as part of the overall planting with 227 Kg ha^{-1} of a pelletized 10:10:10 mix. No additional fertilizer was applied during the experiment.

The plots were examined after 42 weeks (13 September 1991), after 47 weeks (18 October 1991), and after 81 weeks (11 June 1992). At 42 weeks, the number of inflorescences per hill in each plot was counted. At both 42 and 81 weeks after planting the number of culms in each of two randomly selected hills per row in rows 2–9 in plots B–F was determined. Rows 1 and 10 were excluded to minimize edge effects, and a total of 16 hills/plot was counted. In plot G, one random hill in rows 8–23 was assessed (for a total of 16 hills).

After 47 weeks, root samples (*ca.* 50 cm long) were collected from three randomly selected hills in each of the plots. In addition, four root samples were collected from the commercially planted area adjacent to plot G. Soil samples were analysed for the presence of spores of AMF. Root samples were cleared, stained (Koske and Gemma, 1989), and examined for the presence of arbuscular mycorrhizae. The percentage of the length of roots colonized by AMF was estimated using the line-intercept method (Giovannetti and Mosse, 1980).

The results of the field trial were examined using one-way ANOVA. Means were separated using Duncan's multiple range test. Percentage data underwent arcsin transformation before analysis. A significance level of $P = 0{\cdot}05$ was used for all comparisons.

2.3. GREENHOUSE INOCULATION TRIALS

Beach plum (*Prunus maritima*, Rosaceae) is a 3–4 m tall shrub that colonizes relatively early successional sand dunes along the U.S. North Atlantic coast. In the Cape Cod dunes, seedlings successfully establish in older sites in which *A. breviligulata* has gone into decline from lack of sand accretion, but invasion by species intolerant to burial by sand is of frequent occurrence (Koske and Gemma, 1992). This species was selected for study because previous field observations of the consistent presence of mycorrhizae in *P. maritima* suggested that it is an obligately mycotrophic species (Koske and Gemma, 1992). After a 48-hr incubation in moist peat at room temperature and a 4-month stratification at 4°C, seeds of *P. maritima* (F. W. Schumacher Co., Sandwich, MA) were germinated in a greenhouse mist bed in a peat:perlite (1:1) mix. One seedling (*ca.* 2 cm tall) was transplanted to each of 10 plastic containers (Deepots®, Steuwe and Sons, Corvallis, OR) measuring 6·5 × 25 cm and containing 560 ml of a 1:1 mix of coarse gravel and Terra-Green®, a calcined clay (Oil Dri Corp., Chicago, IL). The pH of the gravel was 5·3. The gravel was pasteurized by steam (90°C × 2 hrs) and was set aside for 2 weeks before being combined with the Terra-Green and used for plants. After mixing, the gravel:Terra-Green mix contained 3.5 ppm NO_3, 55 ppm P, and 53 ppm K. The pH of this mix was adjusted to 6·5 with lime and checked with a pH meter using a 1:2 soil:water slurry. Because the clay carrier of the inoculum raises the pH, the pH was measured after the inoculum was added.

To each of five pots was added 11·0 g of inoculum of the AM fungus *Glomus intraradices* Schenck and Smith (Nutrilink®, NPI, Salt Lake City, UT) consisting of

TABLE 1. Incidence of arbuscular mycorrhizae in rooted cuttings from the Cape May Plant Materials Center

Species (cultivar)	m/n*
Ammophila breviligulata	
(PMC 90–470 72)	1/3
(PMC 90–470 73)	3/3
(PMC 90–470 85)	3/3
(PMC 90–470 71)	0/3
(Bogue)	2/3
(Cape)	2/3
(Hatteras)	3/3
Myrica pensylvanica	0/3
Prunus maritima	3/3
Rosa rugosa	3/3
Spartina patens	2/3

* m = no. of plants with arbuscular mycorrhizae; n = no. of plants examined.

spores mixed with an attapulgite clay carrier (1000 spores g^{-1}). Control plants received an equal amount of attapulgite clay (less spores) in place of the inoculum. The inoculum or carrier was dispersed throughout the soil in the Deepot. Plants were grown for 19·5 weeks (11 October 1991–25 February 1992) in a greenhouse with supplemental lighting (16L/8D; 350–1375 $\mu EINm^2 s^{-1}$), watered as required, and each was fertilized bi-weekly with 50 ml of 1/2-strength Hoagland's solution with full strength iron and 1/4-strength P (7·5 ppm) (Hoagland and Arnon, 1950), and 1/4-strength micronutrients (Epstein, 1972). At the conclusion of the experiments, the dry weight of each shoot was measured, and the root systems were cleared, stained and assessed for colonization.

Seaside goldenrod (*Solidago sempervirens*, Asteraceae) is a 1–2 m tall perennial that overwinters as underground rootstock and spreads by seeds and rhizomes. It is a common dune species along much of the U.S. Atlantic coast. The species is an early invader of dunes recently vegetated with beachgrass. The occasional absence of mycorrhizae in specimens collected from sand dunes suggested that the species is facultatively mycotrophic (Koske and Gemma, 1992). Seeds of *S. sempervirens* were obtained from the PMC and germinated in a peat:vermiculite mix (1:1) on a mist bench. One seedling (2 cm tall) was transplanted to each of 16 tapered plastic containers (Cone-tainers®, Steuwe and Sons, Corvallis, OR) measuring 20·7 × 3·8 cm and with a capacity of 165 ml. The Cone-tainers were filled with 165 ml of the 1:1 mix of gravel:Terra-Green described above. Into eight of the Cone-tainers, 5·5 g of Nutrilink were dispersed. Controls were set up using an equal amount of attapulgite clay. Plants were grown under greenhouse conditions as described above. Shoot dry weight and presence of root colonization was measured after 38 weeks growth. The results of the greenhouse experiments were analysed using t-tests and a significance level of $P = 0.05$.

3. Results

3.1. MYCORRHIZAL STATUS OF PLANT MATERIALS USED FOR REVEGETATION

Most planting stock contained AMF in its roots as it arrived from the PMC (Table 1). Roots of only one species (*Myrica pensylvanica*) were not mycorrhizal. Spores of four

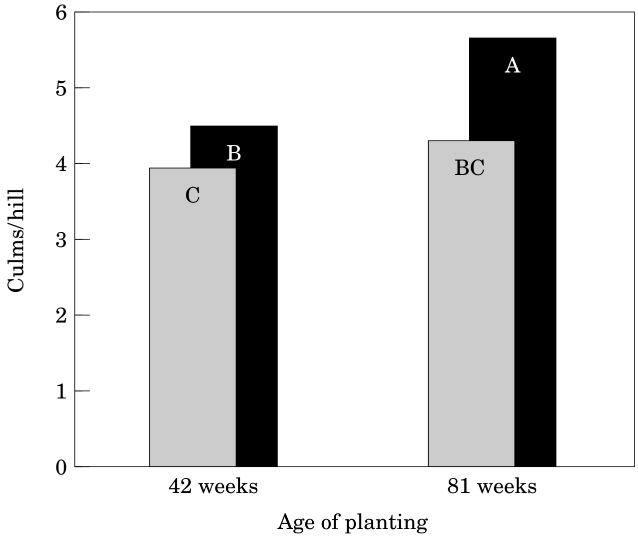

Figure 3. Effect of inoculation on production of culms by plants of *Ammophila breviligulata* grown in dunes at Cape Cod for 42 and 81 weeks. Bars sharing a common letter did not differ significantly ($P>0.05$).

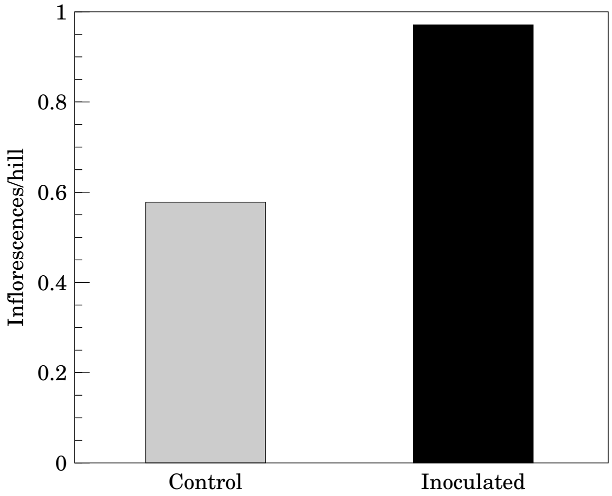

Figure 4. Production of inflorescences by *Ammophila breviligulata* in response to inoculation with native AMF in a field planting. Planting was 42 weeks old at time of sampling. Differences were significant at $P=0.02$.

species of AMF were found in the soil when the roots were sampled: *Gigaspora gigantea* (Nicol. and Gerd.) Gerd. and Trappe, *Glomus clarum*, *Scutellospora erythropa* (Koske and Walker) Walker and Sanders, and *S. pellucida* (Nicol. and Schenck) Walker and Sanders.

3.2. FIELD TRIAL

After 42 weeks, inoculated hills consistently had significantly more culms (14% more) (Figure 3) and inflorescences (67% more) than did control plots (Figure 4). After 81

Table 2. Incidence of arbuscular mycorrhizae in *Ammophila breviligulata* and extent of root colonization 47 weeks after planting

Treatment/plot	m/n*	Percent root length colonized†
Control		
B	3/3	4·2
F	1/3	1·1
G	3/3	6·3
B, F, G combined	7/9	3·9
Inoculated		
C	2/3	4·0
D	2/3	2·9
E	1/3	1·1
C, D, E combined	5/9	2·7
Commercial‡	3/4	4·2

* m = number of plants with VAM; n = number of plants examined.
† percent of total root length in which structures of AMF were present.
‡ plantings made by the commercial planters, adjacent to plot G.

weeks, the difference between the number of culms/hill in the two treatments was greater than at 42 weeks, averaging 31% more in the inoculated culms (Figure 3). When roots systems were examined after 47 weeks, mycorrhizae were present in 78% of the uninoculated plants, in 56% of the inoculated plants, and in 75% of the plants in the adjacent commercial planting (Table 2). Colonization was low (1–6% of root length) in all sites, and no statistically significant differences were present among incidence or extent of colonization.

3.3. GREENHOUSE TRIALS

Seedlings of *P. maritima* grew poorly without AMF, and at the end of the experiment, shoots of inoculated plants weighed 2·8 times more than the controls (Figure 5a). Non-inoculated plants were stunted and their leaves were small, puckered, and red, indicative of P deficiency. None of the control plants had formed mycorrhizae, and all of the inoculated plants had.

Inoculated plants of *Solidago sempervirens* were 62% larger than control plants (Figure 5b), although the difference was not statistically significant. Roots of all control plants were non-mycorrhizal and those of inoculated plants were mycorrhizal.

4. Discussion

Arbuscular mycorrhizal fungi readily invade barren areas of sand dunes, arriving at the site in the planting stock of a variety of species that are planted out as rooted material (e.g. *A. breviligulata*, *P. maritima*, *R. rugosa*, and *S. patens*). Plant species that require AMF for growth and survival thus are able to become established in dune areas that otherwise are lacking sufficient inoculum of AMF. The high incidence of mycorrhizae in the uninoculated field plantings of *A. breviligulata* reflects the occurrence of AMF in the planting stock.

The widespread practice of placing three culms of *A. breviligulata* in each planting hole (Jagschitz and Bell, 1966) increases the chance of the group including at least one

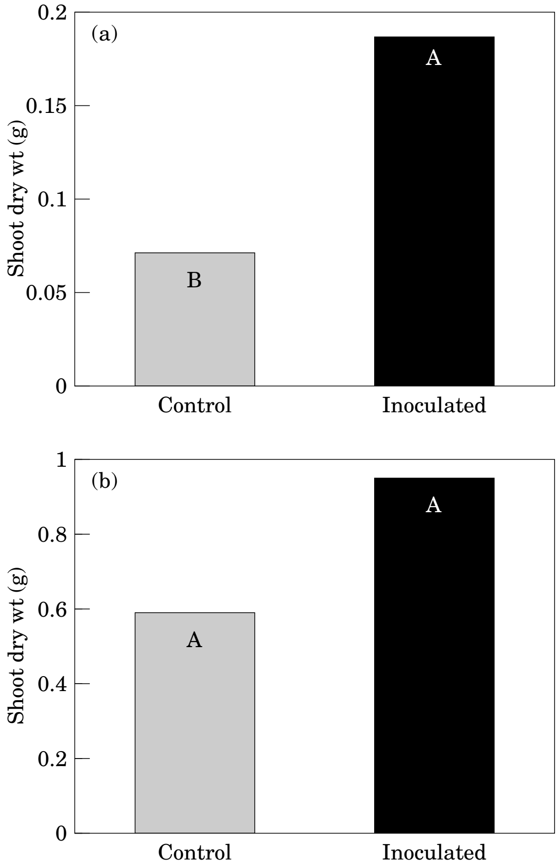

Figure 5. Response of dune species to inoculation with the AMF *Glomus intraradices* in greenhouse experiments. A. Average dry weights of shoots of *Prunus maritima* after 19·5 weeks. B. Average dry weights of shoots of *Solidago sempervirens* after 38 weeks. Bars sharing a common letter did not differ significantly (P>0.05).

individual culm that harbors AMF. A preliminary investigation revealed that 21% of the culms used in the 1990 field planting harbored AMF and were capable of forming mycorrhizae in the absence of added inoculum (Koske and Gemma, 1992). Placement of three culms/hill thus results in a 63% chance that the hill will contain plants that will become mycorrhizal without intentional inoculation. The 63% value is similar to the 78% value observed in the field plantings.

In contrast to the materials used in the field planting, a higher percentage of culms of *A. breviligulata* that were supplied by the PMC contained AMF prior to outplanting (67%). This observation suggested that nursery practices (e.g. fertilization, soil mixes, and pesticides) can influence the extent of mycorrhizal development in *A. breviligulata* as in other species (e.g. Biermann and Linderman, 1983; Koske and Gemma, 1995). Practices that encourage extensive formation of mycorrhizae in *A. breviligulata* are likely to produce planting stock that more frequently carries mycorrhizal fungi and therefore is capable of more rapid establishment (see below).

In the field trial, the addition of AMF to planting holes of *A. breviligulata* stimulated the establishment of the plants, and the benefits were continued throughout the 81-week-long experiment. The greater production of culms and inflorescences in response to inoculation despite the development of mycorrhizae in the control plantings probably reflects the more rapid colonization of new roots as they grow into the soil containing the inoculum. Fungi present in the old roots of the planting stock presumably are slower to produce hyphae that can intercept and invade the rapidly growing new roots that are susceptible to colonization only near the tip (Gemma and Koske, 1989).

In other field studies involving the inoculation of *A. breviligulata* (Gemma and Koske, 1989) and *U. paniculata* (Sylvia, 1989) in dune sites with native AMF, results similar to those reported here were obtained. In both studies, control plants became mycorrhizal because of the presence of AMF in the planting stock, but inoculated plants continued to be more vigorous for 65–80 weeks after planting.

Root colonization of mature plants of *A. breviligulata* from various dune sites in New England typically ranges from *ca.* 40–90% (Koske and Polson, 1984). The low colonization (4–6%) of roots from plants in the field study 47 weeks after planting was less than expected, but improved growth of *A. breviligulata* in response to inoculation in the field has been recorded when colonization extent was as low as 1·5% (Gemma and Koske, 1989).

In addition to ensuring that the newly planted culms soon become mycorrhizal, the early presence of AMF in dune soils allows a network of fungal hyphae to develop sooner (Grime *et al.*, 1987; Read and Birch, 1988; Read, 1989). In dunes at CCNS, more than 6 years elapsed after planting of *A. breviligulata* (not inoculated with AMF) before significant hyphal networks had become established (Koske and Gemma, 1997). Once formed, the network then functions to colonize seedlings of later invading species such a *P. maritima* and *R. rugosa*, species that appear to be absolutely dependent on mycorrhizae (Koske and Gemma, 1992). Thus, plant succession is regulated in part by the presence or absence of AMF at a site (e.g. Miller, 1979; Reeves *et al.*, 1979; Allen and Allen, 1980; Janos, 1980; Dodd *et al.*, 1990a, b; Gemma and Koske, 1990, 1992; Koske and Gemma, 1990).

The ability of uninoculated *S. sempervirens* seedlings to grow well in the greenhouse study reflects their independence from mycorrhizae. This ability (facultative mycotrophy) was further confirmed by the occurrence of both mycorrhizal and non-mycorrhizal specimens the field (Koske and Gemma, 1992). Facultative mycotrophy allows *S. sempervirens* to be among the earliest invaders of dune sites, as seen in the field. Despite its independence from mycorrhizal associations, *S. sempervirens* does not dominate young AMF-poor dunes in part because successful invasion depends on prior occupation of the site by species such as *A. breviligulata* that can ameliorate some of the abiotic stresses (e.g. excessive sand movement, high temperatures, desiccation) that otherwise prevent seedlings from establishing (van der Valk, 1974; Baldwin and Maun, 1983).

The dominance of vegetatively reproducing and rhizomatous species in young dune systems traditionally has been linked to the greater likelihood of these relatively large propagative units (in comparison to seeds and seedlings) to tolerate harsh abiotic conditions during establishment (e.g. high temperatures and drying of the dune surface, greater carbohydrate reserves to allow rapid root growth and ability to grow through deposits of fresh sand) (Maun, 1985; Grubb, 1987). However, the ability of vegetative units to carry AMF with them to P-deficit dune areas may be equally important in the successful colonization of such sites by natural means and in programs concerned with restoration of native ecosystems.

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References

Abe, J-I. P., Masuhara, G. and Katsuya, K. (1994). Vesicular-arbuscular mycorrhizal fungi in coastal dune plant communities. I. Spore formation of *Glomus* spp. predominates under a patch of *Elymus mollis*. *Mycoscience* **35**, 223–238.

Abe, J-I. P. and Katsuya, K. (1995). Vesicular-arbuscular mycorrhizal fungi in coastal dune plant communities. II. Spore formation of *Glomus* spp. predominates under geographically separated patches of *Elymus mollis*. *Mycoscience* **36**, 113–116.

Al-Agely, A. K. and Reeves, F. B. (1995). Inland sand dune mycorrhizae: effects of soil depth, moisture, and pH on colonization of *Oryzopsis hymenoides*. *Mycologia* **87**, 54–60.

Allen, E. B. and Allen, M. F. (1980). Natural re-establishment of vesicular-arbuscular mycorrhizae following strip-mine reclamation in Wyoming. *Wyoming Journal of Applied Ecology* **17**, 139–147.

Baldwin, K. A. and Maun, M. A. (1983). Microenvironment of Lake Huron sand dunes. *Canadian Journal of Botany* **61**, 241–255.

Biermann, B. J. and Linderman, R. G. (1983). Effect of container plant growth medium and fertilizer phosphorus on establishment and host growth response to vesicular-arbuscular mycorrhizae. *Journal of the American Society of Horticultural Science* **108**, 962–971.

Dalpe, Y. (1989). Inventaire et répartition de la flore edomycorrhizienne de dunes et de rivages maritimes du Québec, du Nouveau-Brunswick et de La Nouvellé-Ecosse. *Le Naturaliste Canadien* **16**, 219–236.

Dodd, J. C., Arias, L., Koomen, I. and Hayman, D. S. (1990a). The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem I. The effect of pre-cropping and inoculation with VAM-fungi on plant growth and nutrition in the field. *Plant and Soil* **122**, 229–240.

Dodd, J. C., Arias, L., Koomen, I. and Hayman, D. S. (1990b). The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem II. The effects of pre-crops on the spore populations of native and introduced VAM-fungi. *Plant and Soil* **122**, 241–247.

Epstein, E. (1972). *Mineral Nutrition of Plants: Principles and Perspectives*. New York: Wiley and Sons.

Francis, R. and Read, D. J. (1994). The contribution of mycorrhizal fungi to the determination of plant community structure. *Plant and Soil* **159**, 11–25.

Francis, R. and Read, D. J. (1995). Mutualism and antagonism in the mycorrhizal symbioses, with special reference to impacts on plant community structure. *Canadian Journal of Botany* **73** (Suppl.), S1301–S1309.

Gemma, J. N. and Koske, R. E. (1988). Seasonal variation in spore abundance and dormancy of *Gigaspora gigantea* and in mycorrhizal inoculum potential of a dune soil. *Mycologia* **80**, 211–216.

Gemma, J. N. and Koske, R. E. (1989). Field inoculation of American beachgrass, (*Ammophila breviligulata*) with VA mycorrhizal fungi. *Journal of Environmental Management* **29**, 173–182.

Gemma, J.N. and Koske, R. E. (1990). Mycorrhizae in recent volcanic substrates in Hawaii. *American Journal of Botany* **77**, 1193–1200.

Gemma, J. N. and Koske, R. E. (1992). Are mycorrhizas present in early stages of primary succession? In *Proceedings of Third European Symposium on Mycorrhizas: Mycorrhizas in Ecosystems* (D. R. Read, D. H. Lewis, A. H. Fitter and I. J. Alexander, eds), pp. 183–189. Oxon: CAB International.

Giovannetti, M. (1985). Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. *Transactions of the British Mycological Society* **84**, 679–684.

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* **84**, 489–500.

Giovannetti, M. and Nicolson, T. H. (1983). Vesicular-arbuscular mycorrhizas in Italian sand dunes. *Transactions of the British Mycological Society* **80**, 552–557.

Godfrey, P. J. (1977). Climate, plant response and development of dunes on barrier beaches along the U.S. east coast. *International Journal of Biometeorology* **21**, 203–215.

Godfrey, P. J., Leatherman, S. P. and Zaremba, R. (1979). A geobotanical approach to classification of barrier beach systems. In *Barrier islands from the Gulf of St. Lawrence to the Gulf of Mexico* (S. P. Leatherman, ed.), pp. 99–126. New York: Academic Press.

Grime, J. P., Mackey, J. M. L., Hillier, S. H. and Read, D. J. (1987). Floristic diversity in a model system using experimental microcosms. *Nature* **328**, 420–422.

Grubb, P. J. (1987). Some generalizing ideas about colonization and succession in green plants and fungi. In *Colonization, Succession and Stability* (A. J. Gray, M. J. Crawley, and P. J. Edwards, eds), pp. 31–56. Oxford: Blackwell Scientific Publications.

Harley, J. L. and Smith, S. E. (1983). *Mycorrhizal Symbiosis*. London: Academic Press.

Hewett, D. G. (1970). The colonization of sand dunes after stabilization with marram grass (*Ammophila arenaria*). *Journal of Ecology* **58**, 653–668.

Hoagland, D. R. and Arnon, D. I. (1950). The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347.

Jagschitz, J. A. and Bell, R. S. (1966). American beachgrass (establishment–fertilization–seeding). Bulletin 383. Agricultural Experiment Station, University of Rhode Island, Kingston, Rhode Island.

Janos, D. P. (1980). Mycorrhizae influence tropical succession. *Biotropica* **12**, 56–64.

Jehne, W. and Thompson, C. H. (1981). Endomycorrhizae in plant colonization on coastal sand dunes at Cooloola, Queensland. *Australian Journal of Ecology* **6**, 221–230.

Koske, R. E. (1975). *Endogone* spores in Australian sand dunes. *Canadian Journal of Botany* **53**, 668–672.

Koske, R. E. (1987). Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. *Mycologia* **79**, 55–68.

Koske, R. E. (1988). Vesicular-arbuscular mycorrhizal fungi of some Hawaiian dune plants. *Pacific Science* **42**, 217–229.

Koske, R. E. and Gemma, J. N. (1989). A modified procedure for staining roots to detect V-A mycorrhizas. *Mycological Research* **92**, 486–488.

Koske, R. E. and Gemma, J. N. (1990). VA mycorrhizae in vegetation of the Hawaiian coastal strand: evidence for co-dispersal of fungi and plants. *American Journal of Botany* **77**, 466–474.

Koske, R. E. and Gemma, J. N. (1992). Restoration of early and late successional dune communities at Province Lands, Cape Cod National Seashore. Tech. Rep. NPS/NARURI/NRTR-92/03. Coop. NPS Studies Unit, University of Rhode Island, Narragansett Bay Campus. Narragansett, Rhode Island.

Koske, R. E. and Gemma, J. N. (1995). VA mycorrhizal inoculation of Hawaiian plants: a conservation technique for endangered tropical species. *Pacific Science* **49**, 181–191.

Koske, R. E. and Gemma, J. N. (1996). Arbuscular-mycorrhizal fungi in Hawaiian sand dunes: Island of Kauai. *Pacific Science* **50**, 36–45.

Koske, R. E. and Gemma, J. N. (1997). Mycorrhizae and succession in plantings of beachgrass in sand dunes. *American Journal of Botany* **84**, 118–130.

Koske, R. E. and Halvorson, W. L. (1981). Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Canadian Journal of Botany* **59**, 1413–1422.

Koske, R. E. and Halvorson, W. L. (1989). Mycorrhizal associations of selected plant species from San Miguel Island, Channel Islands National Park, California. *Pacific Science* **43**, 32–40.

Koske, R. E. and Polson, W. R. (1984). Are VA mycorrhizae required for sand dune stabilization? *BioScience* **34**, 420–424.

Koske, R. E., Sutton, J. C. and Sheppard, B. R. (1975). Ecology of *Endogone* in Lake Huron sand dunes. *Canadian Journal of Botany* **53**, 87–93.

Maun, M. A. (1985). Population biology of *Ammophila breviligulata* and *Calamovilfa longifolia* on Lake Huron sand dunes. I. Habitat, growth form, reproduction and establishment. *Canadian Journal of Botany* **63**, 113–124.

Miller, R. M. (1979). Some occurrences of vesicular-arbuscular mycorrhiza in natural and disturbed ecosystems of the Red Desert. *Canadian Journal of Botany* **57**, 619–623.

Nelson, C. E. (1987). The water relations of vesicular-arbuscular mycorrhizal systems. In *Ecolphysiology of Mycorrhizal Plants* (G. R. Safir, ed.), pp. 71–91. Boca Raton: CRC Press.

Newsham, K. K., Fitter, A. H. and Watkinson, A. R. (1995). Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* **83**, 991–1000.

Nicolson, T. H. (1959). Mycorrhiza in the Gramineae. I. Vesicular-arbuscular endophytes, with special reference to the external phase. *Transactions of the British Mycological Society* **42**, 421–438.

Nicolson, T. H. (1960). Mycorrhiza in the Gramineae. II. Development in different habitats, particularly sand dunes. *Transactions of the British Mycological Society* **43**, 132–145.

Nicolson, T. H. and Johnston, C. (1979). Mycorrhiza in the Gramineae III. *Glomus fasciculatus* as the endophyte of pioneer grasses in a maritime sand dune. *Transactions of the British Mycological Society* **72**, 261–268.

Puppi, G. and Riess, S. (1987). Role and ecology of VA mycorrhizae in sand dunes. *Angewandte Botanik* **61**, 115–126.

Ranwell, D. S. (1972). *The Ecology of Salt Marshes and Sand Dunes*. London: Chapman and Hall.

Read, D. J. (1989). Mycorrhizas and nutrient cycling in sand dune ecosystems. *Proceedings of the Royal Society of Edinburgh* **96B**, 89–110.

Read, D. J. and Birch, C. P. P. (1988). The effects and implications of disturbance of mycorrhizal mycelial systems. *Proceedings of the Royal Society of Edinburgh* **94B**, 13–24.

Reeves, F. B., Wagner, D., Moorman, T. and Kiel, J. (1979). The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. *American Journal of Botany* **66**, 6–13.

Rose, S. L. (1988). Above and below-ground community development in a marine sand dune ecosystem. *Plant and Soil* **109**, 215–226.

Strahler, A. N. (1966). *A Geologist's View of Cape Cod*. New York: Natural History Press.

Sturmer, S. L. and Bellei, M. M. (1994). Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. *Canadian Journal of Botany* **72**, 359–363.

Sutton, J. C. and Sheppard, B. R. (1976). Aggregation of sand-dune soil by endomycorrhizal fungi. *Canadian Journal of Botany* **54**, 326–333.

Svenson, H. K. and Pyle, R. W. (1979). *The Flora of Cape Cod*. Massachusetts: Cape Cod Museum of Natural History.

Sylvia, D. M. (1986). Spatial and temporal distribution of vesicular-arbuscular mycorrhizal fungi associated with *Uniola paniculata* in Florida foredunes. *Mycologia* **78**, 728–733.

Sylvia, D. M. (1989). Nursery inoculation of sea oats with vesicular-arbuscular mycorrhizal fungi and outplanting performance of Florida beaches. *Journal of Coastal Research* **5**, 747–754.

Sylvia, D. M. and Burks, J. N. (1988). Selection of a vesicular-arbuscular mycorrhizal fungus for practical inoculation of *Uniola paniculata*. *Mycologia* **80**, 565–568.

Sylvia, D. M. and Will, M. E. (1988). Establishment of vesicular-arbuscular mycorrhizal fungi and other microorganisms on a beach repleinishment site in Florida. *Applied and Environmental Microbiology* **54**, 348–352.

Sylvia, D. M., Jarstfer, A. G. and Vosátka, M. (1993). Comparisons of vesicular-arbuscular mycorrhizal species and inoculum formulations in a commercial nursery and on diverse Florida beaches. *Biology and Fertility of Soils* **16**, 139–144.

van der Valk, A. G. (1974). Environmental factors controlling the distribution of forbs on coastal foredunes in Cape Hatteras National Seashore. *Canadian Journal of Botany* **52**, 1057–1073.

Walker, C., Mize, C. W. and McNabb, H. S. (1982). Populations of endogonaceous fungi at two locations in central Iowa. *Canadian Journal of Botany* **60**, 2518–2529.

Whatley, M. E. (1988). *Common Trailside Plants of Cape Cod National Seashore*. Massachusetts: Eastern National Park and Monument Association.

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