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Interaction of Rhizosphere Bacteria, Fertilizer, and Vesicular-Arbuscular Mycorrhizal Fungi with Sea Oats†

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Plants must be established quickly on replenished beaches in order to stabilize the sand and begin the dune-building process. The objective of this research was to determine whether inoculation of sea oats (*Uniola paniculata* L.) with bacteria (indigenous rhizosphere bacteria and N_2 fixers) alone or in combination with vesicular-arbuscular mycorrhizal fungi would enhance plant growth in beach sand. At two fertilizer-N levels, *Klebsiella pneumoniae* and two *Azospirillum* spp. did not provide the plants with fixed atmospheric N; however, *K. pneumoniae* increased root and shoot growth. When a sparingly soluble P source ($CaHPO_4$) was added to two sands, *K. pneumoniae* increased plant growth in sand with a high P content. The phosphorus content of shoots was not affected by bacterial inoculation, indicating that a mechanism other than bacterially enhanced P availability to plants was responsible for the growth increases. When sea oats were inoculated with either *K. pneumoniae* or *Acaligenes denitrificans* and a mixed *Glomus* inoculum, there was no consistent evidence of a synergistic effect on plant growth. Nonetheless, bacterial inoculation increased root colonization by vesicular-arbuscular mycorrhizal fungi when the fungal inoculum consisted of colonized roots but had no effect on colonization when the inoculum consisted of spores alone. *K. pneumoniae* was found to increase spore germination and hyphal growth of *Glomus deserticola* compared with the control. The use of bacterial inoculants to enhance establishment of pioneer dune plants warrants further study.

Accelerated costal erosion threatens private and public property in many areas of the world. Such erosion is considered critical in Florida because of continuing high investments in shoreline development and the revenues generated by the tourist industry (10). Lost sand is replaced with material of compatible physical properties which is shaped to the desired beach profile and planted with pioneer species, such as sea oats (*Uniola paniculata* L.) and panic grass (*Panicum* spp.), to enhance beach stability and begin the dune-building process. The major factors limiting establishment and early, vigorous growth of dune plants in the face of environmental extremes are infertility and the poor moisture-holding capacity of coarse replenishment materials (4, 17). Rhizosphere microorganisms may allow beach grasses to overcome these environmental extremes (1).

The association between N_2-fixing bacteria and grasses is well documented (33). Total plant nitrogen (N) gains due to N_2 fixation by *Azotobacter* spp. have been reported for *Ammophila arenaria* grown in dune sand with an exogenous carbon (C) source (1). Either low (2, 28) or high (R. Ralph, Ph.D. dissertation, University of Delaware, Newark, 1978) rhizosphere nitrogenase activity has been reported for several grasses growing in unamended sand. Significant enhancement of growth, but not of plant-N content, often occurs following root inoculation with asymbiotic N_2-fixing bacteria (14, 18, 22). These growth increases may be due to microbially produced phytohormones which affect plant root morphology and nutrient uptake (43).

The ability of rhizosphere bacteria to solubilize phosphorous (P) may be important in East Coast dune sands of Florida, where plant-available P is low. Louw and Webley (23) found that the majority of over 100 bacterial isolates from the rhizosphere of *Avena sativa* could solubilize several forms of insoluble calcium phosphate minerals in vitro. Plants inoculated with rhizosphere bacteria and grown in media with $CaHPO_4$ as the sole P source had increased P contents compared with noninoculated plants (12).

Vesicular-arbuscular mycorrhizal (VAM) fungi have increased the growth and P content of dune grasses in greenhouse and field studies (31, 40, 41). Dual inoculations with VAM fungi and bacteria (N_2 fixers or P solubilizers) have been done with a variety of plants, but no such studies have been reported for organisms from the beach environment. Results of previous dual inoculations were mixed; some workers found no effect of dual inoculation on plant growth (8, 9, 35), while others reported a synergistic plant response (5–7, 25, 32, 34).

Preliminary experiments in our laboratory indicated that bacteria affect the germination of spores of VAM fungi and subsequent root colonization. Mugnier and Mosse (27) suggested that *Streptomyces orientalis* produces volatile compounds that stimulate germination of spores of *Glomus mosseae*. Germination of spores and establishment of root infection using spores alone can be difficult with some VAM fungi unless bacteria are present (26, 27).

Greenhouse experiments were established to determine whether inoculation of sea oat seedlings with bacteria alone or VAM fungi and bacteria together would enhance the growth and nutrient status of sea oats grown in sand. In vitro and greenhouse experiments were also conduced to determine whether the presence of bacteria affected spore germination and early hyphal growth of VAM fungi.

MATERIALS AND METHODS

Bacterial and fungal isolates. Five bacterial isolates were chosen on the basis of their ability to fix atmospheric N_2 in vitro (42; M. E. Will, Ph.D. dissertation, University of Florida, Gainesville, 1988). The isolates were (i) *Bacillus polymyxa* (identified by Microbial I.D., Inc., Newark, Del.) isolated from the rhizosphere of sea oats from Atlantic

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Beach, Fla., (ii) *Alcaligenes denitrificans* (identified by Microbial I.D.) isolated from the rhizosphere of sea oats from Miami Beach, Fla., (iii) *Azospirillum lipoferum* USA5b (Pullman, Wash.; soil), (iv) *Azospirillum brasiliense* JM125A2 (Gainesville, Fla.; millet), and (v) *Klebsiella pneumoniae* Beijing. The *Azospirillum brasiliense*, *Azospirillum lipoferum*, and *K. pneumoniae* isolates were from J. Milam (Microbiology and Cell Science Department, University of Florida, Gainesville) and were included to assess the effects of bacteria with known high potential rates of N_2 fixing on growth of sea oats. In addition, a combination of two non-N_2-fixing, gram-negative bacteria (T3E3 and T3E10) isolated from the rhizosphere of sea oats in established dunes at Atlantic Beach, Fla., were tested.

The VAM fungi were isolated from the rhizosphere of sea oats on established dunes at Anastasia State Recreation Area (ASRA) at St. Augustine Beach, Fla., by D. M. Sylvia. The fungi were *Glomus deserticola* Trappe, Blosse and Menge (isolate S305) and an undescribed *Glomus* sp. (isolate S328). These were found to be, overall, the most abundant species of VAM fungi at ASRA and in established dunes at Atlantic Beach, Fla. (38, 42).

Soil analysis. Pasteurized ASRA and Miami Beach sands were analyzed before the experiments for particle size, pH, electrical conductivity, Ca, N, and Mehlich I-extractable P by the Analytical Research Laboratory, University of Florida, and for soil-equilibrium-solution P by the method described by Sylvia (39).

Calcium phosphate solubilization. A laboratory study was conducted to determine the ability of the *K. pneumoniae*, *Azospirillum lipoferum*, and *Alcaligenes denitrificans* to solubilize $CaHPO_4$ in vitro. Bacterial cultures were grown in liquid nutrient broth to log phase, washed twice in NaCl (8 g liter^{-1}), and suspended in sterile water. A dilution series was prepared from the washed cells, and 1 ml of each dilution was added to 15 ml of a sterile $CaHPO_4$-containing medium in Petri dishes. The medium contained in milligrams liter $^{-1}$: sucrose, 2,000; $CaCl_2$, 10; $MgSO_4 \cdot 7H_2O$, 200; $NaMoO_4$, 20; KNO_3, 250; NH_4NO_3, 160; Fe, 5.6 (Fe-EDTA); $CaHPO_4$, 1,000. It also contained 10 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.). Plates (eight per bacterial strain) were observed daily for 10 days for zones of clearing around the bacterial colonies, indicating $CaHPO_4$ solubilization (19).

Bacterial inoculation, N fertilization, and plant growth. Sea oat seeds collected from plants on sand dunes at ASRA were germinated and grown for 10 days in vermiculite. Four seedlings were then transplanted to each of 620-ml Deepots (J. M. McConkey & Co., Inc., Sumner, Wash.) containing 600 ml of pasteurized (70°C for 4 h) ASRA sand. Immediately prior to transplanting, 1 ml of washed cells (10^7 CFU ml^{-1}) or sterile water (control) was placed in the transplanting hole. For the first N experiment, bacterial inoculants were *K. pneumoniae*, *Azospirillum brasiliense*, *Azospirillum lipoferum*, *B. polymyxa* and *Alcaligenes denitrificans*. For the second N experiment, inoculants were *K. pneumoniae* and *Alcaligenes denitrificans*. The cultures were prepared for use as inoculum as described above for the $CaHPO_4$ test. The plants were fertilized at the time of planting and every 14 days thereafter with 20 ml of a 1:10 dilution of modified Hoagland solution (16) containing in milligrams liter^{-1}: K, 23.5 (KCl, KH_2PO_4); P, 3.1 (KH_2PO_4); Ca, 20 ($CaCl_2$); Mg, 4.8 ($MgSO_4$); S, 6.4 ($MgSO_4$); Fe, 5.6 (Fe-EDTA); and micronutrients (solution A). One-half of the plants in each inoculation treatment received N in the fertilizer solution at a rate of 2 mg liter^{-1} (NH_4NO_3), while the other half received no fertilizer N. The plants were arranged in a greenhouse in a completely randomized block design with 10 replicates per treatment. The first N experiment lasted 63 days, during which the mean minimum and maximum temperatures were 22 and 33°C, respectively, and the mean maximum photosynthetic photon flux density (PPFD) was 1,232 μmol m^{-2} s^{-1}. The second N experiment lasted 70 days during which the mean minimum and maximum temperatures were 22 and 29°C, respectively, and the mean maximum PPFD as 1,407 μmol m^{-2} s^{-1}.

Bacterial inoculation and phosphorus uptake. The experimental pots were set up as described for the N experiments, using *K. pneumoniae*, *Azospirillum lipoferum*, and *Alcaligenes denitrificans*. Deepots contained pasteurized sand, with 50 kg of P ha^{-1} as $CaHPO_4$ mixed in by hand prior to planting. Plants were fertilized with 20 ml of fertilizer solution containing 2 mg of N liter^{-1}, no P, and 20 mg of K liter^{-1} at the time of planting and every 14 days thereafter. The plants were arranged in a greenhouse in a completely randomized block design with 10 replicates per treatment. The first P experiment used ASRA sand and lasted 90 days during which the mean minimum and maximum temperatures were 23 and 31°C, respectively, and the mean maximum PPFD was 1,766 μmol m^{-2} s^{-1}. The second P experiment used Miami Beach sand and lasted 100 days, during which the mean minimum and maximum temperatures were 19 and 27°C, respectively, and the mean maximum PPFD was 1,285 μmol m^{-2} s^{-1}. During the final 30 days of growth, the plants in the second experiment were illuminated with 900 μmol of supplemental light m^{-2} s^{-1} for 14 h day^{-1} from metal halide lights. The temperature at plant height under the lights was 31°C.

Spores and colonized sea oat roots as inocula. Two experiments were conducted using a mixture of *G. deserticola* (S329) and *Glomus* sp. (S328) spores. Approximately 50 spores in 2 ml of sterile water were placed at a depth of 5 cm below the surface of 600 ml of pasteurized ASRA sand in Deepots 10 days prior to planting with four sea oat seedlings which had been germinated and grown for 10 days in vermiculite. Spores, stored in partially dried sand at 5°C for 16 months, were collected from a pot culture by wet sieving and sucrose centrifugation (11). In a third experiment, Deepots containing either ASRA or Miami Beach pasteurized sand were inoculated with 20 cm of washed, chopped sea oat roots with associated hyphae and intraradicle spores 10 days prior to planting seedlings that had been grown for 21 days in a potting mix of vermiculite and peat by a commercial grower (Horticultural Systems, Parrish, Fla.). The sea oat roots had 10% of their length colonized with the mixed *Glomus* culture. Controls in all three experiments received, 10 days prior to planting, 2 ml of water that had been used to wash the inoculum onto a 45 μm-pore-size screen.

Immediately prior to transplanting, 1 ml (10^7 CFU) of washed bacterial cells of *K. pneumoniae* (experiment 1), *Alcaligenes denitrificans* (experiment 2), a mixture of *K. pneumoniae* and *Azospirillum lipoferum* (experiment 3), or sterile water (all controls) was placed in the transplanting hole. Plants inoculated with spores were fertilized at the time of planting and every 14 days thereafter with 20 ml of a 1:10 dilution of modified Hoagland solution containing in milligrams liter^{-1}: N, 20 (NH_4NO_3); P, 0.05 (KH_2PO_4); and K, 23.5 (KCl, KH_2PO_4). Plants inoculated with colonized roots were fertilized as described above, except that the N rate was 10 mg liter^{-1} and the P rate was either 0 or 0.05 mg liter^{-1} (19.8 or 23.4 mg of K liter^{-1}, respectively). The Deepots were arranged in a greenhouse in a completely

TABLE 1. Effects of N fertilization, across inoculation treatment, on growth of sea oat seedlings in sand from ASRA[a]

N rate (mg liter^{-1})	RDM (g)	TRL (cm)	HTT (cm)	SDM (g)	Shoot-N concn (g kg^{-1})	Total shoot N (mg)	Shoot-P concn (g kg^{-1})	Total shoot P (mg)
Expt 1								
0	0.033 B	299 B	16 B	0.037 B	8.71 B	0.28 B	2.23 A	0.08 B
2	0.128 A	602 A	29 A	0.102 A	12.91 A	1.31 A	1.14 B	0.13 A
Expt 2								
0	0.018 B	350 B	35 B	0.035 B	16.82 B	0.57 B	1.52 A	0.06 B
2	0.044 A	607 A	96 A	0.096 A	32.04 A	3.21 A	1.11 B	0.11 A

[a] Each value for shoot and root growth represents the mean of at least eight replicates, while values for nutrient contents represent the mean of three replicates. An N treatment mean with the same letter as the control mean is not significantly different from the control mean.

randomized block design with 10 replicates of each treatment. Each experiment was run for 100 days, with mean minimum and maximum temperatures of 19 and 30°C, respectively, and a PPFD of 1,470 μmol m^{-2} s^{-1}.

Effect of *K. pneumoniae* on spore germination: in vitro experiments. An experiment was conducted to determine whether *K. pneumoniae* produced volatile compounds that could affect germination of VAM fungal spores. Two opposing quarters of divided, sterile, polystyrene Petri dishes (100 by 15 mm) received 4 ml of 1.5% water agar (Difco). The two remaining wells received 4 ml of 1.5% nutrient agar (Difco). Spores of *G. deserticola* were surface disinfested (2 min in 52.5 g of sodium hypochlorite liter^{-1}) and then rinsed three times with 20 ml of sterile water under a gentle vacuum. Five spores were placed on each quadrant containing water agar. One-half of the plates received 0.01 ml of nutrient broth (Difco) containing *K. pneumoniae* (10^6 CFU) in the quadrants containing nutrient agar. The plates were wrapped with Parafilm (American Can Co., Greenwich, Conn.) and incubated at 28°C in the dark. The number of spores germinated (germ tube length of at least 5 μm) on each day (noncumulative) was determined after 2, 4, 5, 6, 7, 8, and 9 days. After 9 days, the greatest length of hyphal extension from each germinated spore was measured. Means and standard errors of nine replicates were determined.

A second experiment was conducted as described above except that there were six plates per treatment. In this case, each water agar quadrant contained 10 spores of *G. deserticola* and plates were examined after 4, 6, and 8 days.

Effect of *K. pneumoniae* on spore germination: greenhouse experiments. Sea oat seeds were germinated in vermiculite, and 10 days later they were transplanted (one seedling per tube) to 77-ml Pinecell tubes (Ray Leach Cone-Tainer Nursery, Canby, Oreg.) containing pasteurized ASRA sand. Spores of *G. deserticola* were surface disinfested and placed in sterile water at a density of 5 spores ml^{-1}. Gelman polysulfone filters (pore size, 0.2 μm, diameter, 25 mm), under gentle suction in a filter apparatus, received 1 ml of the spore suspension. One-half of the filters also received 1 ml (10^7 CFU) of washed *K. pneunoniae* cells. At transplanting, the filters were folded twice and placed approximately 1 cm to the side of roots of sea oats seedlings, at a depth of 3 cm. Plants were fertilized at planting with 10 ml of fertilizer solution containing 0.3 mg of P liter^{-1}, 23 mg of K liter^{-1}, and 10 of mg N liter^{-1}.

Five randomly sampled replicates of each treatment were harvested after 2, 4, 6, 28, 48, 68, and 88 days. The filters were removed and stained with 0.5 g of trypan blue kg^{-1}, and the total number of spores and number of spores germinated on each filter were recorded. Roots were cleared and stained for observation of VAM fungi. Root fresh mass and total and colonized root length were measured at 48, 66, and 88 days.

A second experiment was set up and analyzed as described above. Seven randomly sampled replicates of each treatment were harvested, and spore germination was determined after 5, 8, 28, 66, and 80 days. Root fresh mass and total and colonized root length were measured after 66 and 80 days. The mean minimum and maximum temperatures for both experiments were 19 and 27°C, respectively, and the mean maximum PPFD was 1,433 μmol m^{-2} s^{-1}.

Sampling and analysis. For all greenhouse experiments, the height of the tallest tiller (HTT) in each pot was measured, adhering sand was washed gently from the root mass, and roots were weighed. Shoots were dried at 60°C for 48 h, weighed, and shredded by hand. For N analysis, shoots were digested by the Kjeldahl method as modified by Nelson and Sommers (29) and analyzed for N with an Alpkem Rapid Flow Analyzer. For P analysis, shoots were digested by using the sealed-chamber method of Anderson and Henderson (3), and P was determined on a Jarrel-Ash model 9000 inductively coupled argon plasma spectrometer. A portion of the root mass (0.5 g), subsampled for colonization by VAM fungi, was cleared in 1.0 g of KOH liter^{-1} overnight and stained with 0.5 g of trypan blue liter^{-1}. Total and VAM-colonized root lengths were estimated by the grid-line-intersect method (30). Percentage data were subjected to an arcsine transformation for analysis. Data were subjected to analysis of variance, and differences in treatment means ($P \leq 0.05$) were evaluated by orthogonal contrasts (37).

RESULTS

Soil analysis. The ASRA sand was of finer texture and had higher NO_3-N (0.37 mg kg^{-1} versus a nondetectable amount), water-extractable P (0.08 versus 0.01 mg liter^{-1}), and Mehlich I-extractable P (633 versus 3 mg kg^{-1}) than did the Miami Beach sand. The pHs of the ASRA and Miami Beach sands were 9.1 and 9.6, respectively. The mineralogy of the fine silt and clay fractions of the ASRA sand was dominated by a brushitelike ($CaHPO_4 \cdot 2H_2O$) compound (W. G. Harris, personal communication).

Calcium phosphate solubilization. Zones of $CaHPO_4$ solubilization were visible around the colonies of all bacterial strains within 96 h. *K. pneumoniae* grew rapidly and had clear zones about 4 mm wide around each colony at 10 days , and *Azospirillum lipoferum* and *Alcaligenes denitrificans* colonies were surrounded by 1- to 2-mm wide and less than 1-mm-wide clear zones, respectively.

Bacterial inoculation, N fertilization, and plant growth. In the first experiment, fertilizer N resulted in increases in root dry mass (RDM), total root length (TRL), HTT, shoot dry

TABLE 2. Effect of bacterial root inoculation on growth of sea oat seedlings for 90 days in ASRA sand amended with $CaHPO_4$[a]

Inoculation treatment	RDM (g)	TRL (cm)	HTT (cm)	SDM (g)
Control	0.071 A	386 A	29 A	0.135 A
K. pneumoniae	0.093 B	552 B	30 B	0.166 B
Azospirillum lipoferum	0.076 A	372 A	29 A	0.132 A
Alcaligenes denitrificans	0.077 A	378 A	28 A	0.143 A

[a] Each value represents the mean of at least 19 replicates. Microbial treatment means with the same letter as the control mean are not significantly different from the control mean.

mass (SDM), and percent and total shoot N (Table 1). There was an interactive effect of N and microbial inoculation on shoot growth. At the lower fertilizer-N level, plants inoculated with *K. pneumoniae* or T3E3 plus T3E10 had greater SDM than did controls (0.041, 0.038, and 0.031 g, respectively), while at the higher fertilizer-N level, only plants inoculated with *K. pneumoniae* had significantly greater SDM compared with the controls (0.153 and 0.086 g, respectively). Control plants had a higher shoot N concentration than did inoculated plants (19.6 g kg^{-1} versus an average for all bacterial treatments of 9.3 g kg^{-1}), and plants inoculated with *K. pneumoniae* had higher RDM than did controls, regardless of N fertilization (0.103 versus 0.096 g). There were high coefficients of variation (up to 90%) associated with these data. Variability in growth within varieties of wild grasses has been recognized as a problem in seed and forage production (13) and likely contributed to the variability in these experiments.

In the second experiment, nitrogen fertilization resulted in increases over controls in RDM, TRL, HTT, and SDM of 150, 73, 100, and 150%, respectively (Table 1). Shoot-N concentrations and total N content were increased by 90 and 500%, respectively. Shoot-P concentration was reduced 28% by N fertilization; however, there was a concurrent 100% increase in total P.

Bacterial inoculation and phosphorus uptake. In sand from ASRA, inoculation with *K. pneumoniae* resulted in increases in RDM, TRL, HTT, and SDM of 31, 43, 3, and 23%, respectively, over controls (Table 2). Shoot P and N were not affected by inoculation, with mean contents of 0.24 and 1.95 mg, respectively. In sand from Miami Beach, microbial inoculation had no effect on RDM, TRL, HTT, SDM, total shoot N, shoot-P concentration, and total shoot P (mean values were 0.084 g, 653.5 cm, 35.9 cm, 0.254 g, 2.23 mg, 1.4 g kg^{-1}, and 0.35 mg, respectively). Inoculation with *K. pneumoniae* or *Alcaligenes denitrificans* decreased shoot-N concentration by 3.2 and 2.9 g kg^{-1}, respectively, from the control value of 10.1 g kg^{-1}.

Spores and colonized sea oat roots as inocula. There were no differences between inoculation treatments and controls with regard to RDM, TRL, HTT, SDM, and total shoot P in either experiment using spores alone as the inoculum. Overall mean values were 0.043 and 0.026 g for RDM, 633 and 251 cm for TRL, 28 and 27 cm for HTT, 0.16 and 0.10 g for SDM, and 0.12 and 0.09 mg for total shoot P for the two experiments. Plant-N data were insufficient for statistical analysis. In the second experiment, the P concentrations in shoots of plants inoculated with *Glomus* spores alone or in combination with *Alcaligenes denitrificans* were increased over that in control plants (0.10, 0.11, and 0.07 g kg^{-1}, respectively). Root colonization remained low in treatments receiving the VAM fungi (means of 10% in the first experiment and 21% in the second experiment) and was not affected by the presence of the bacteria.

When colonized roots were used as inoculum in the Miami Beach sand, phosphorus fertilization increased RDM and shoot-N concentrations over controls (0.04 versus 0.02 g and 16.2 versus 14.4 g kg^{-1}, respectively). In plants receiving P, colonization with VAM fungi was increased by the combined bacterial and fungal inoculation compared with fungal inoculation alone (56 versus 41%). Regardless of fertilizer P level, total P and shoot-P concentrations were increased by inoculation with the bacterial combination compared with those in the controls (16.2 g kg^{-1} and 0.32 mg of P versus 7.9 g kg^{-1} and 0.09 mg of P, respectively). In the ASRA sand, fertilization with P resulted in increased root colonization (42 versus 35%), RDM (0.06 versus 0.04 g), TRL (402 versus 311 cm), HTT (33 versus 27 cm), and total shoot N (0.20 versus 0.18 mg), compared with the no-P control. Inoculation with VAM fungi resulted in greater RDM than did the control treatment (0.06 and 0.05 g, respectively), regardless of P-fertilization level. In this sand, dual inoculation had no effect on colonization by VAM fungi. Control plants receiv-

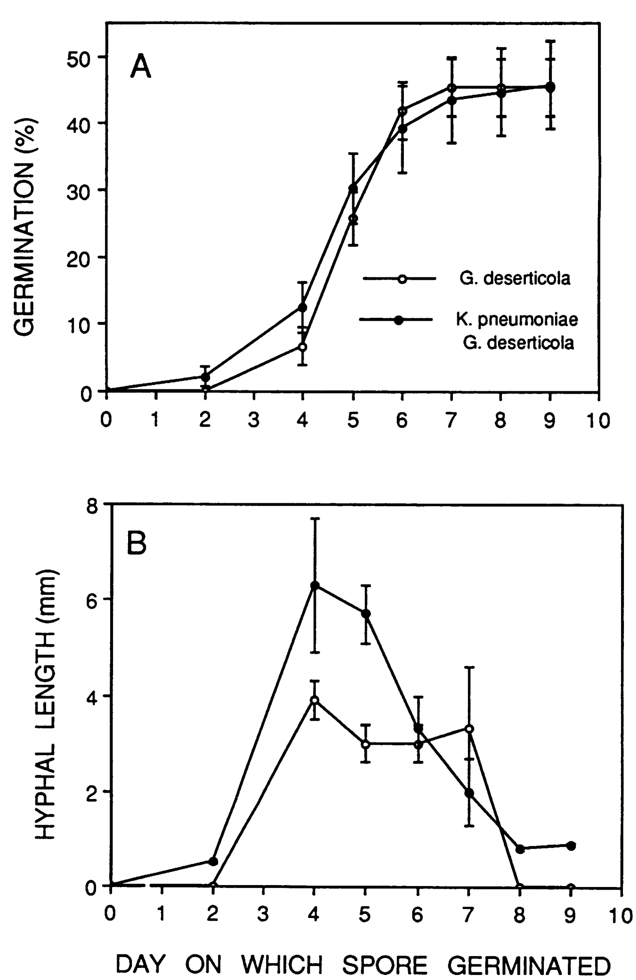

FIG. 1. Percent germination (A) and length of hyphal extension measured on day 9 (B) of *G. deserticola* spores with and without *K. pneumoniae*. Datum points in panel A represent means of nine replicates ± standard errors of the means. Datum points in panel B represent 0, 5, 15, 12, 4, 0, and 0 spores for *G. deserticola* and 2, 8, 18, 3, 1, and 1 spores for *K. pneumoniae-G. deserticola* on days 2, 4, 5, 6, 7, 8, and 9, respectively.

ing no fertilizer P had higher shoot-N concentration (14.2 g kg^{-1}) than plants inoculated with the bacteria alone (10.3 g kg^{-1}), the fungi alone (9.9 g kg^{-1}), or both (10.7 g kg^{-1}). Dual-inoculated plants had increased shoot-N concentration compared with the controls, regardless of fertilizer-P level (12.3 and 8.3 g kg^{-1}, respectively). Inoculation with either the bacteria alone or in combination with the VAM fungi resulted in higher total shoot N than that in controls plants, regardless of fertilizer-P level (1.71, 2.17, and 1.19 mg of N, respectively).

Effect of *K. pneumoniae* on spore germination: in vitro experiments. *K. pneumoniae* in compartments next to those containing *G. deserticola* spores had no significant effect on the number of spores that germinated (Fig. 1A). However, hyphal extension away from the germinated spores, as measured on day 9, was increased by the presence of bacteria (Fig. 1B). In the second experiment, the effect of *K. pneumoniae* on hyphal extension was even more pronounced: hyphae from spores which germinated on day 4 in the presence of *K. pneumoniae* grew 5 mm, as measured on day 9; spores without bacteria grew only 0.9 mm.

Effect of *K. pneumoniae* on spore germination: greenhouse experiments. In the first experiment, spore germination at 28 and 48 days was increased by *K. pneumoniae*, compared with that of spores on filters without the bacteria (Fig. 2). The presence or absence of the bacteria did not affect plant RDM and TRL or root colonization by the VAM fungus. Mean values were 0.01 g for RDM, 70 cm for TRL, and 0% for colonization at 48 days; 0.02 g for RDM, 90 cm for TRL, and 1% for colonization at 68 days; and 0.02 mg for RDM, 100 cm for TRL, and 2% for colonization at 88 days.

In the second experiment, spore germination at 66 and 80 days was increased by *K. pneumoniae* (48 versus 39% and 60 versus 42%, respectively), compared with that of spores on filters without the bacteria. The presence or absence of the bacteria again did not affect plant RDM and TRL or root colonization by VAM fungi. Mean values were 0.01 g for RDM, 42 cm for TRL, and 6% for colonization at 66 days and 0.01 g for RDM, 58 cm for TRL, and 11% for colonization at 80 days.

DISCUSSION

We found no evidence of significant plant-N or -P increases due to inoculation with bacteria. The fact that N was lower in the inoculated plants than in control plants indicates that plant growth enhancement by *K. pneumoniae* was not due to increased N availability resulting from microbial N_2 fixation and supports the idea that root-associated N_2 fixation in temperate climates is seriously limited by carbohydrate availability (21). Decreased plant-N content may have resulted from microbial competition for limited N available in the soil. The fact that the P content of shoots was not affected by bacterial inoculation following addition of insoluble P suggests that a mechanism other than bacterially enhanced P availability was responsible for the plant growth increases seen with *K. pneumoniae*. Root-induced lowering of the rhizosphere pH or root-produced P-chelating organic acids may have been responsible for making P more available to all experimental plants (24). It is also possible that P was sequestered in the roots inoculated with bacteria; root sample sizes were too small for nutrient analyses.

Increases in growth of sea oat seedlings outplanted to Miami Beach (40), resulting from inoculation with VAM fungi, were not seen here. Although it is likely that fungal hyphae growing into the sand aided in water and nutrient uptake, the plants were not under severe water or nutrient stress in these greenhouse experiments. There was no consistent evidence for a synergistic effect of dual inoculation with *K. pneumoniae* or *Alcaligenes denitrificans* and VAM fungi on sea oat growth. Root colonization by VAM fungi was lower in experiments in which spores alone were used than when colonized root inoculum was used. This may have been because the latter contains fungal propagules other

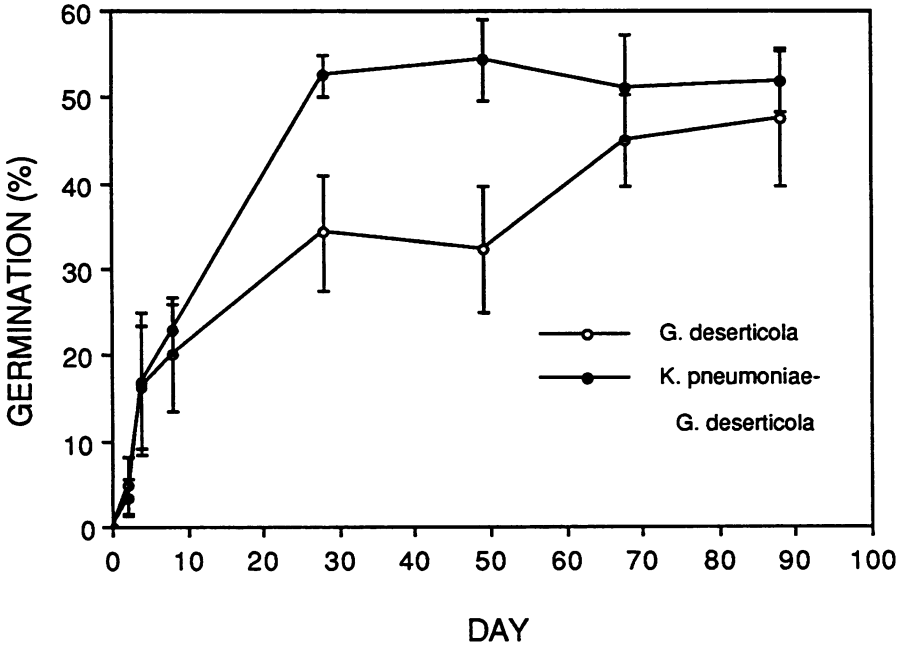

FIG. 2. Percent germination of *G. deserticola* spores with and without *K. pneumoniae* in a greenhouse experiment. Datum points represent means of five replicates ± standard errors of the means.

than spores, allowing more rapid formation of mycorrhizae (15).

Colonization of roots was enhanced by bacteria in the Miami Beach sand. These findings are in agreement with the results of Rao et al. (35) for *G. mosseae*, *Azospirillum brasiliense*, and barley and of Pacovsky et al. (32) for *Glomus fasciculatum*, *Azospirillum brasiliense*, and sorghum. Bacteria may affect root cell walls, thereby increasing susceptibility of the plant tissue to fungal penetration. *Azospirillum brasiliense* produces pectolytic enzymes in vitro which soften root cell walls in the soil (44). This mechanism was suggested by Mosse (26) for increased colonization of roots of several plants by *Endogone* sp. in the presence of *Pseudomonas* sp. It was also suggested by Meyer and Linderman (25) to explain enhanced colonization of clover roots by an unidentified VAM fungus in the presence of *Pseudomonas putida*.

Azcon (5) found that the presence of bacteria did not affect spore germination of VAM fungi, but did increase hyphal length. Significant stimulation of hyphal extension also occurred in our study. *K. pneumoniae* may produce a volatile substance which stimulates hyphal extension. However, spore germination was enhanced in our experiments when bacteria and spores were physically contiguous on the filters in the soil, suggesting involvement of a nonvolatile, diffusible substance. Additional work is needed to identify volatile and nonvolatile compounds released by *K. pneumoniae* and to clarify their effects on germination of spores of VAM fungi. Slower spore germination in soil compared with that on agar may have been the result of suppression by soil microflora, among other environmental factors (20, 36).

The results of these experiments indicate the need for further evaluation of the use of bacterial inoculants to enhance growth of pioneer dune grasses. It is apparent that rhizosphere bacteria can enhance or suppress VAM symbiosis, although little is known about the mechanisms of these effects. Additional research is warranted to better understand how these organisms interact in the rhizospheres of plants.

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