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Growth promotion of the seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp.

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Abstract Inoculation of the oilseed halophyte *Salicornia bigelovii* Torr. with eight species of halotolerant bacteria, grown in seawater-irrigated pots under environmental conditions native to the plant's habitat, resulted in significant plant growth promotion by the end of the growing season, 8-11 months later. Statistical analysis demonstrated that inoculation with *Azospirillum halopraeferens,* a mixture of two *Azospirillum brasilense* strains, a mixture of *Vibrio aestuarianus* and *Vibrio proteolyticus,* or a mixture of *Bacillus licheniformis* and *Phyllobacterium* sp. significantly increased plant height and dry weight at the end of the season. Some of the bacterial strains also increased the number of side branches and the size of the spikes. The bacteria did not affect the number of seeds or their weight. Inoculation with the mangrove cyanobacterium *Microcoleus chthonoplastes* had no effect on plant foliage variables. At the end of the growing season, the N and protein content of the plant foliage was significantly reduced by bacterial inoculation; however, the N and protein content of seeds significantly increased. The P content in foliage increased significantly in plants treated with all the bacteria except *M. chthonoplastes,* whereas the total lipid content of foliage increased significantly only when plants were inoculated with a mixture of *A. brasilense* strains or with *M. chthonoplastes*. In three inoculation treatments palmitic acid in seeds significantly increased and linoleic acid significantly decreased. This study demonstrates the feasibility of using bacteria to promote the growth of halotolerant plants cultivated for forage and seed production in proposed seawater irrigated agriculture.

Key words *Azospirillum* . *Oilseed* halophytes
Plant-growth-promoting bacteria - *Salicornia*
Seawater-irrigated agriculture

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Introduction

Species of the shrub-like halophytic weed, *Salicornia,* are grown widely in climates ranging from temperate to tropical (Jefferies et al. 1981; Rey et al. 1990; Benito and Onaindia 1991). Monocultures and mixed cultures of *Salicornia* and other salt marsh weeds occur commonly in coastal salt marshes and inland salt pans where stands often exceed 10,000 plants m^2 (Ellison 1987). Although the plants can tolerate freshwater to some extent, NaCI is required for normal growth (Ayala and O'Leary 1995).

Salicornia bigelovii Torr. is an annual, leafless, fastgrowing, succulent halophyte found along the coastline of Sonora and Baja California in Mexico (RodriguezMedina et al. 1998). Because *S. bigelovii* produces oilseeds and a high yield of foliage (Glenn et al. 1998), it is being evaluated as a new forage and oilseed crop for saltwater- and seawater-irrigated agriculture in the coastal deserts of Mexico (Troyo-Dieguez et al. 1994) and the USA, as are other *Salicornia* species in other countries (Glenn et al. 1991, 1998). Despite its high salt content, animals fed moderate levels of *Salicornia* gained as much weight as those whose diet included hay or other terrestrial weeds (Swingle et al. 1996).

Inoculation of crop plants with plant-growth-promoting bacteria (PGPB), is a contemporary agricultural practice used to improve crop yields. PGPBs include biocontrol strains that benefit plants indirectly by inhibiting fungal pathogens (Ogushi et al. 1997), species such as *Azospirillum* sp. that enhance plant growth directly (Bashan and Holguin 1997), or endophytic bacteria that have both direct and biocontrol plant-growth promoting capacities (Hallmann et al. 1997).

Bacteria with confirmed plant-growth-promoting capabilities have not yet been isolated from *Salicornia* plants, although some potential PGPB strains (e.g. N_2-fixing *Azotobacter* or PO_4^{3-}-solubilizing bacteria), untested as yet, were detected in the rhizosphere of these plants (Diab and Al-Gounaim 1984).

Some species of *Azospirillum*, a known terrestrial PGPB, are halotolerant (Hartmann and Zimmer 1994) and can survive well in seawater when inoculated on mangrove seedling roots (Puente et al. 1999). In addition, several potential marine PGPBs (N_2-fixing and PO_4^{3-}-solubilizing bacteria) were isolated from mangrove seedlings (Vazquez et al. 2000). Bacterial mixtures are often more effective at promoting plant growth than monocultures (Bashan and Holguin 1997).

The aim of this study was to evaluate the response of *S. bigelovii* to inoculation with several of the above PGPB under a seawater-irrigation regime and environmental growth conditions resembling *Salicornia's* natural habitat.

Materials and methods

Growth conditions for plants and bacteria

S. bigelovii. seeds were harvested from wild plants on the shore at El Comitan, Baja California Sur, Mexico (24°1'N, 110°2'W) and were stored in hermetically sealed 1-1 glass containers at ambient temperature for 7 days. Before inoculation, the seeds were treated as follows: 1875 seeds, of the same volume and size by a flotation test, were disinfected by agitating in 3% $NaC10_3$ for 5 min and then rinsed with sterile tap water.

The seeds were inoculated with the following bacteria:

1. The terrestrial halotolerant bacterium *Azospirillum halopraeferens* AU10 [tolerant to 3% salt (Reinhold et al. 1987); capable of growing in seawater in the presence of mangrove seedlings (Puente et al. 1999); donated by B. Reinhold-Hurek, Max-Planck Institute, Marburg, Germany].
2. A mixture of *Azospirillum brasilense* Cd [DSM 7030 Braunschweig, Germany; salt tolerance of 2% NaCl (Holguin and Bashan 1996); also capable of colonizing mangrove roots in seawater (Puente et al. 1999) and *A. brasilense* Sp-245 (unknown salt tolerance; donated by J. Döbereiner, EMBRAPA, Brazil)].
3. *Vibrio aestuarianus* (a marine N_2-fixing bacterium) combined with the marine PO_4^{3-}-solubilizing bacterium *Vibrio proteolyti*cus (Vazquez et al. 2000).
4. A mixture of *Phyllobacterium* sp. (a marine N_2-fixing bacterium) and the marine PO_4^{3-}-solubilizing bacterium *Bacillus licheniformis* (Vazquez et al. 2000).
5. *Microcoleus chthonoplastes* B-1 [a marine N_2-fixing cyanobacterium (Toledo et al. 1995)].

The N_2-fixing bacteriaV. *aestuarianus* and *Phyllobacterium sp.*, both isolated from young black mangrove plants *[Avicennia germinans (L.)* Stern (Holguin et al. 1992)] were donated by G. Holguin, CIB, Mexico. All bacteria, excluding *Azospirillum spp.*, were isolated from mangrove rhizosphere. Because of the intensive maintenance of the experiment (daily irrigation with measured seawater for almost a year), single bacterial treatments showing no effect on plant growth in preliminary tests were not used as controls for treatments involving mixed inoculants.

Bacteria were grown in the following media: *A. brasilense* strains in OAB medium (Bashan et al. 1993), *A. halopraeferens* in OAB supplemented with 0.5% NaCl (Reinhold et al. 1987), V. *proteolyticus* and *B. licheniformis* in SRSMl medium (Vazquez et al. 2000), V. *aestuarianus* and *Phyllobacterium* sp. in BMDA medium containing: 4 g malic acid l^{-1}; 4 g citric acid l^{-1},; 2 g D-glucose l^{-1};2 g D-mannitol l^{-1}; 1 g myoinositol l^{-1}; 23.4 g NaCI l^{-1}; 1.5 g KCl l^{-1}; 24.6 g $MgSO_4 \cdot 7H_20$ l^{-1}; 2.9 g $CaCl_2 \cdot 2H20$ l^{-1}; 75 mg K_2HPO_4 l^{-1}; 28 mg $FeSO_4 \cdot 7H_20$ l^{-1}; 1 µg biotin l^{-1}; 2 µg piridoxine l^{-1}; 40 µg $NaMoO_4 \cdot 2H_2O$ l^{-1}; 47 µg $MnSO_4 \cdot H_20$ l^{-1}; 56 µg H_3B0_3 l^{-1}; 1.6 µg $CuSO_4 \cdot 5H_20$ l^{-1}; 47 µg $ZnSO_4 \cdot 7H_20$ l^{-1}; and 10 ml glycerol; final pH, 7.2 (Holguin 1998).

Using a standard inoculation technique developed for *Azospirillum* sp. (Puente and Bashan 1993), seeds were inoculated with 10^6 colony forming units (cfu) ml^{-1} of bacterial cells in the logarithmic phase (Bashan 1986a) suspended in saline solution (0.85% NaCl). *M. chthonoplastes* was inoculated according to Bashan et al. (1998). When two species of bacteria were mixed, the concentration of each was 10^6 cfu ml^{-1}. Inoculated seeds were immediately sown at 0.5-cm depth in 1.5-1 white opaque pots (12 cm wide, 20 cm depth) containing 250 g sterile (two consecutive 15min periods in an autoclave) commercial potting mixture with a high organic matter level (Sunshine Mix no. 4, special fine; Fisons Horticulture, Mississauga, Ontario). Twenty-five seeds were sown in each pot, and after germination (3-5 days) all seedlings in each pot were retained. Plants were inoculated a second time after 60 days with 10^6 cfu pot^{-1} of bacterial cells immobilized in alginate beads (3-mm-diameter beads). The bead inoculant was produced as described by Bashan (1986b) from commercial alginate (3500 centipois; Sigma).

To standardize the inoculum, bacteria were counted in the liquid and bead inoculants by a standard plate-count method on nutrient agar (Difco, Detroit, Mich.) for *A. brasilense* (Bashan et al. 1993), on OAB supplemented with 0.5% NaCl for *A. halopraeferens* (Reinhold et al. 1987), and on modified SRSMl medium for PO_4^{3-}-solubilizing bacteria (Vazquez et al. 2000). Root colonization was not measured during the experiment to avoid damage to the growing plants, and because a reliable immunoidentification method was available only for *A. brasilense* Cd and not for the novel PGPBs. During the course of this long experiment, pots became contaminated with airborne bacteria; however, because the growth area was terrestrial and because *Azospirillum sp.* has not been isolated thus far from the grounds at the Center of Biological Research of the Northwest (M. E. Puente, personal communication), we assumed these contaminants were of terrestrial origin and would not have an impact on this study. No attempt was made to identify or to quantify the contaminants.

To facilitate seedling survival, to acclimate seeds for seawater irrigation, and avoid salt-stress shock (Troyo-Dieguez and Breceda Solis-Camara 1992), the pots were irrigated with fresh tap water for the first 15 days, then with 50% seawater (diluted in the same freshwater) for 15 days, and 75% seawater for an additional 15 days, and finally with 100% seawater. Plants were manually irrigated daily with 100 ml filtered, sterile seawater (Puente et al. 1999) and were grown in a protected ambient-temperature net house on elevated metal net beds (to avoid contamination by bacteria released from the pot's drainage hole) for up to 11 months (15 April 1998 to 22 March 1999) until completion of the life cycle. To avoid possible contamination, the pots were scattered randomly throughout the entire greenhouse (20 x 8 m, length x width). During the experimental period, the ambient temperature within the net house varied from 24:12 °C (day: night) in January and February to 36:23 °C (day :night) in August and September. Because the net house was less than 100 m from the bay shore where wild *S. bigelovii* plants were growing, the climatic conditions during the course of the experiment were almost identical to those experienced by wild populations. The plants were not affected by any pest or disease; therefore pesticides were not applied.

Analyses of plant variables

After 90 days of cultivation, plants were thinned to leave only 1 plant pot^{-1} for the next 6-8 months. The dry weight of the extracted plants

was measured. The remaining plants continued to grow in the pots until they completed their life cycle (i.e. until plants produced seeds and dried despite irrigation with seawater). The time to complete the life cycle was influenced by bacterial treatment (see Results section).

At the end of the life cycle the following plants variables were measured: whole plant dry weight [dried at 80 °C for 24 h in a forced-draught oven (model VWR 1680, Sheldon Manufacturing, Cornelius, Ore.)], plant height, number of branches, number of spikes and their length, number of seeds (after manual extraction), number of aborted seeds, seed dry weight, dry weight of 100 seeds, total N and protein content in plants and in seeds [automatic micro-Kjeldahl after digestion (Digestion system 12.1009, and Kjeltec auto 1030 analyzer; Tecator, Höganäs, Sweden)], total lipids (Bligh and Dyer 1959), and P content in plants and seeds (Kitson and Mellon 1944 as modified by Hach Co., Loveland, Colo.). Determination of four fatty acid profiles in seeds (palmitic, linoleic, linolenic, and oleic acids) were done by injecting 0.4 µl samples into a gas chromatograph (HP 5890 series II, Workstation HP Chemstation software, USA) using a Supelcowax 10 column (30 m length x 0.53 mm inner diameter) operating at an initial temperature of 150°C for 15 min and then increasing at 0.5 °C min^{-1} for a total of 180 min until reaching a final temperature of 230 °C. Gas flow rates were: 2 ml N_2 min^{-1}, 30 ml H_2 min^{-1}, and 300 ml air min^{-1} using the following lipid standards obtained from Sigma: palmitic acid methyl ester (ME) (16:0), 20%; stearic acid ME (18:0), 20%; oleic acid (18:1), 20%; linolenic acid ME (18:2), 20%; and linolenic acid ME (18:3), 20%. Results are expressed as percentages of total fatty acids.

Experimental design and statistical analyses

Each plant treatment was replicated 10 times, where a single pot served as one replicate. Replicates were placed randomly throughout the greenhouse. Because of the intensive labour involved, and to avoid contamination from the other bacterial inoculants used, each inoculation was done on separate days and therefore had its own control (uninoculated plants). At the end of the experiment, each group of similarly treated plants was compared to its own control. Additionally, all control values were combined and subjected to statistical analysis against each treatment. Student's t-test and one-way ANOVA were carried out using Statistica software (Statsoft, Tulsa, Okla.). The actual P values are given for statistically significant different treatments. Data in percentages were transformed to arcsin values before analysis. SEs were calculated for all treatments.

Results

Effect of inoculation on growth period of *Salicornia* plants

Under the experimental conditions, uninoculated *Salicornia* plants grew for 188-231 days to the end of their natural life cycle when, as annuals, they died and dried (Table 1). Inoculation with bacteria extended the growth period significantly to 234-332 days (Table 1). The extension of the growth period was smallest for plants inoculated with a mixture of A. *brasilense* strains (14.3%) and longest for those inoculated with a mixture of *Phyllobacterium* sp. and *B. licheniformis* (58.9%) (Table 1).

Effect of inoculation on *Salicornia* plants 90 days after inoculation

The average dry weight of plants at 90 days after planting ranged from 0.25 to 0.58 g $plant^{-1}$. Only inoculation with the diazotrophic cyanobacteria *M. chthonoplastes* significantly increased ($P \leq 0.05$) plant dry weight over uninoculated plants (by 70%). Inoculation with a mixture of *Phyllobacterium* sp. and *B. licheniformis* significantly reduced plant dry weight (by 46%) and the other inoculation treatments had no significant effect. The water content of all plants whether inoculated or not was similar and ranged from 78-84% of their total weight.

Effect of inoculation on morphological variables of *Salicornia* plants

By the end of the growing season, bacterial inoculation affected plant size. All bacterial treatments yielded visibly larger plants (Fig. 1) and, except for inoculation with the cyanobacteria *M. chthonoplastes*, all bacterial treatments significantly increased plant height over uninoculated plants. The smallest increase (+ 16%) was found in plants inoculated with a mixture of *A. brasilense* strains, whereas other treatments yielded increases of 47-53% (Fig. 2A).

The dry weight of inoculated plants at the end of the growing season was significantly greater than that of uninoculated plants. Plants inoculated with a mixture of *A. brasilense* strains showed the smallest increase in dry weight (+ 44%) whereas those inoculated with *A. halopraeferens* showed the greatest increase (+ 102%). However the largest mass production of plants was produced following inoculation with either of the two mixtures of N_2-fixing bacteria and PO_4^{3-}-solubilizing bacteria, averaging 4 g $plant^{-1}$ (Fig. 2B).

Salicornia does not produce leaves, therefore the number of side branches was used to assess plant growth promotion. The average number of branches ranged from 14 to 53 $plant^{-1}$. The

Table 1 Length of the growth cycle[a] (days) for *Salicornia bigelovii* plants inoculated with different bacteria

Treatment	*Azospirillum halopraeferens*	*Azospirillum brasilense* Cd plus Sp-245	*Vibrio aestuarianus* plus *Vibrio proteolyticus*	*Phyllobacterium sp* plus *Bacillus licheniformis*	*Microcoleus chthonoplastes*
Noninoculated	227	231	204	209	188
Inoculated	332	264	320	332	234

[a] Growth period between germination and natural death of the plant

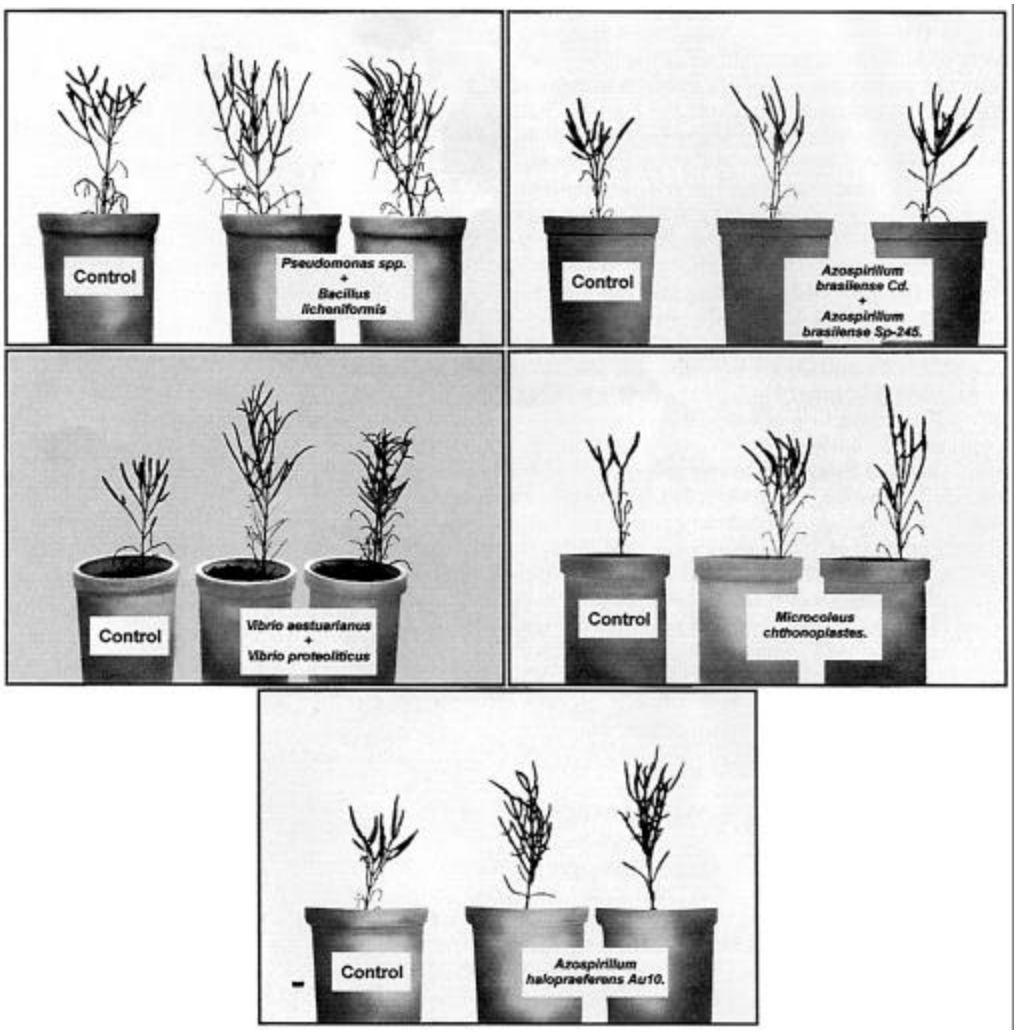

Fig. 1 Effect of inoculation with several plant-growth-promoting bacteria on *Salicornia bigelovii* plants after growth in the greenhouse for 6 months and daily irrigation with seawater. *Bar* = 1 cm

largest increase in branch number was in plants inoculated with a mixture of *Vibrio* spp. (118%) and with *A. halopraeferens* (84%) as compared to uninoculated control plants. The other three bacterial treatments had no significant effect on this variable (Fig. 2C).

Although the number of spikes per plant varied greatly (from 5 to 26 plant^{-1}), none of the bacterial treatments significantly increased the number of spikes per plant compared to uninoculated plants. However, the size of the spikes changed significantly as a result of bacterial inoculation. Spike size increased following inoculation with a mixture of N_2-fixing bacteria and PO_4^{3-}-solubilizing bacteria and with *A. halopraeferens* (by 34-73%). The spikes significantly decreased in size when the plants were inoculated with the cyanobacteria *M. chthonoplastes*, and did not change as a result of inoculation with *A. brasilense* (Fig. 2D). Interestingly, bacterial treatments that yielded the greatest increase in plant dry weight also yielded spikes with more aborted flowers (up to 23 flowers spike^{-1}; data not shown). This had no effect on the number and weight of seeds per plant.

For each of the four foliage variables, all uninoculated control values were combined and analysed by **ANOVA** against each of the values for inoculated plants. The differences between noninoculated control and inoculated values were statistically significant and were identical to the differences between single inoculated plants and noninoculated control plants prepared at the same time (data not presented).

The number of seeds varied greatly among plants, from an average 250-640 seeds plant^{-1}. Consequently, the average total dry weight of seeds per plant also varied, from 0.15 to 0.45 g plant^{-1}. None of the bacterial treatments increased the number of seeds or their weight. The dry weight of 100 seeds was similar in all the treatments whether inoculated or not, and was on average 0.07 g 100 seeds^{-1}. The weight of 100 seeds in the *A. halopraeferens* treatment was 0.0626±0.016 g; in the *A. brasilense* Cd plus *A. brasilense* Sp 245 treatment, 0.0691 ±0.010 g; in the *V. aestuarianus plus V. proteolyticus* treatment, 0.0567±0.013 g; in the *B. licheniformis plus Phyllobacterium sp.* treatment, 0.0580±0.018 g; and in the *M. chthonoplastes* treatment, 0.0737 ± 0.017 g.

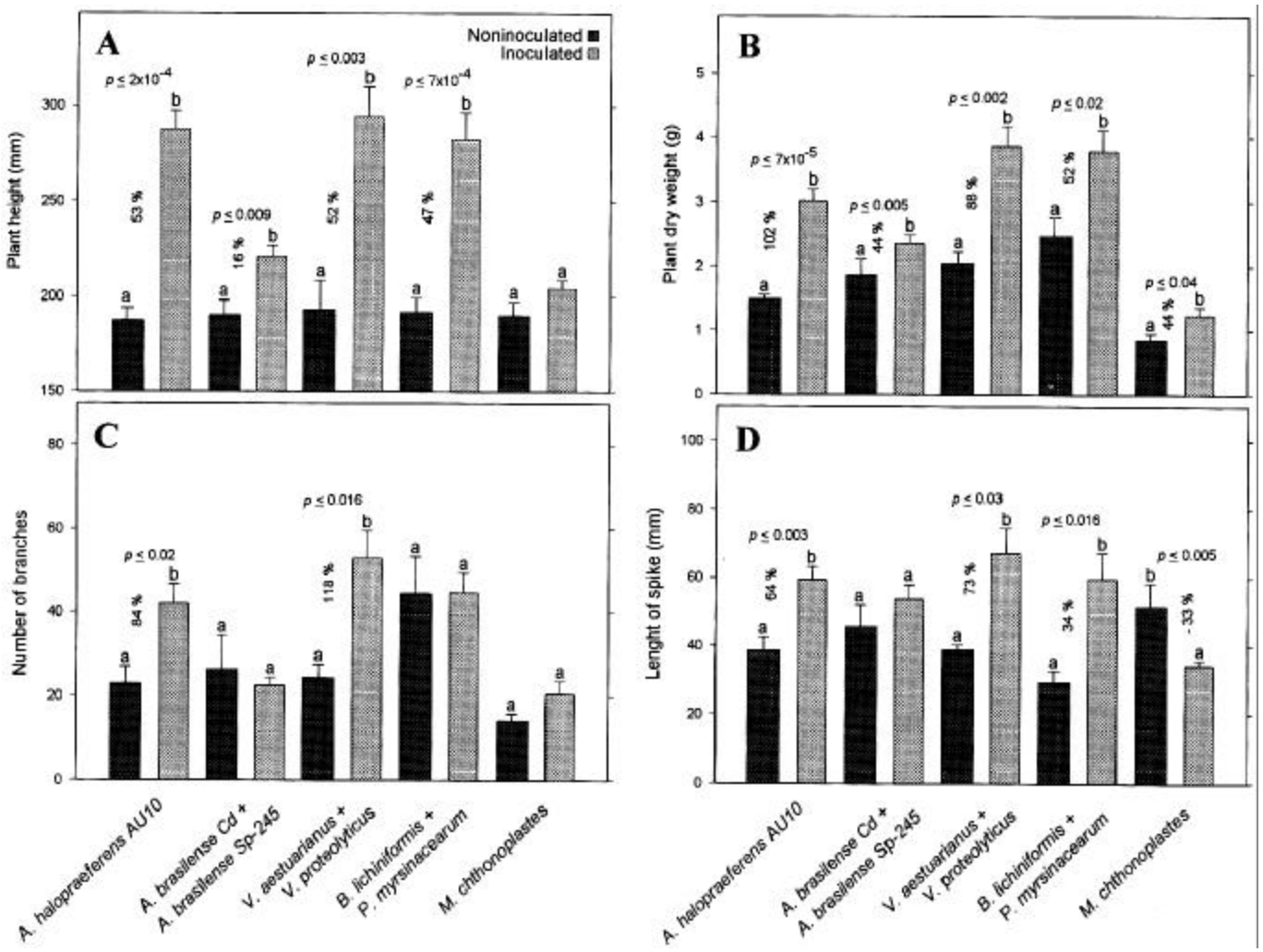

Fig. 2A-D Effect of inoculation with several plant-growth-promoting bacteria on various morphological characteristics of *Salicornia bigelovii* plants at the end of the growing season. **A** Plant height, **B** Dry weight, **C** number of branches, **D** length of spike. Pairs of values (inoculated and uninoculated treatments) that differed significantly at the P level denoted above the pair, are indicated by different lower case letters. Vertical numbers above each pair indicate a significant increase (or decrease; %) in inoculated plants as compared to uninoculated plants. Bars represent SE

Effect of inoculation on the N, protein, P, and lipid contents and the composition of fatty acids of *Salicornia* plants and seeds at the end of the growing season

At the end of the growing season, all bacterial treatments significantly reduced the total N (Fig. 3A) and protein content (data not shown) of plant foliage. At the same time, the N content of seeds significantly increased following bacterial treatment to almost 4 times the original N content, from <1% to almost 4% of plant dry weight (Fig. 3B). Similarly, the protein content in seeds significantly increased four- to five-fold (from 31.3 to 217 mg g^{-1} seeds) for *A. halopraeferens* inoculation. The four bacterial treatments, but not the cyanobacteria treatment, significantly increased the P content of *S. bigelovii* foliage (Fig. 3C). Total P in seeds was not measured because not enough seeds were available for the analysis. The total lipid content in seeds varied with bacterial treatment. Although inoculation with both *A. brasilense* strains or cyanobacteria significantly increased the lipid content, inoculation with the two *Vibrio* strains and with *A. halopraeferens* decreased the lipid content (Fig. 3D). Among the four fatty acids reported for *Salicornia* seeds (Glenn et al. 1991), a difference was detected in the levels of linoleic and palmitic acids. The three inoculation treatments (*A. halopraeferens, V. aestuarianus plus V. Proteolyti*cus, and *Phyllobacterium sp. plus B. licheniformis)* showed that the percentage of palmitic acid significantly increased at the expense of linoleic acid. The other two fatty acids (linolenic and oleic acids) were present only in small quantities (< 2% of the total fatty acids of the seeds) (Table 2).

Discussion

Seawater agriculture is defined as growing salt-tolerant crops on land using water pumped from the sea for irrigation (Boyko 1967). Though a substantial portion of the world's land is suitable for saltwater irrigation, i.e. coastal deserts could be irrigated

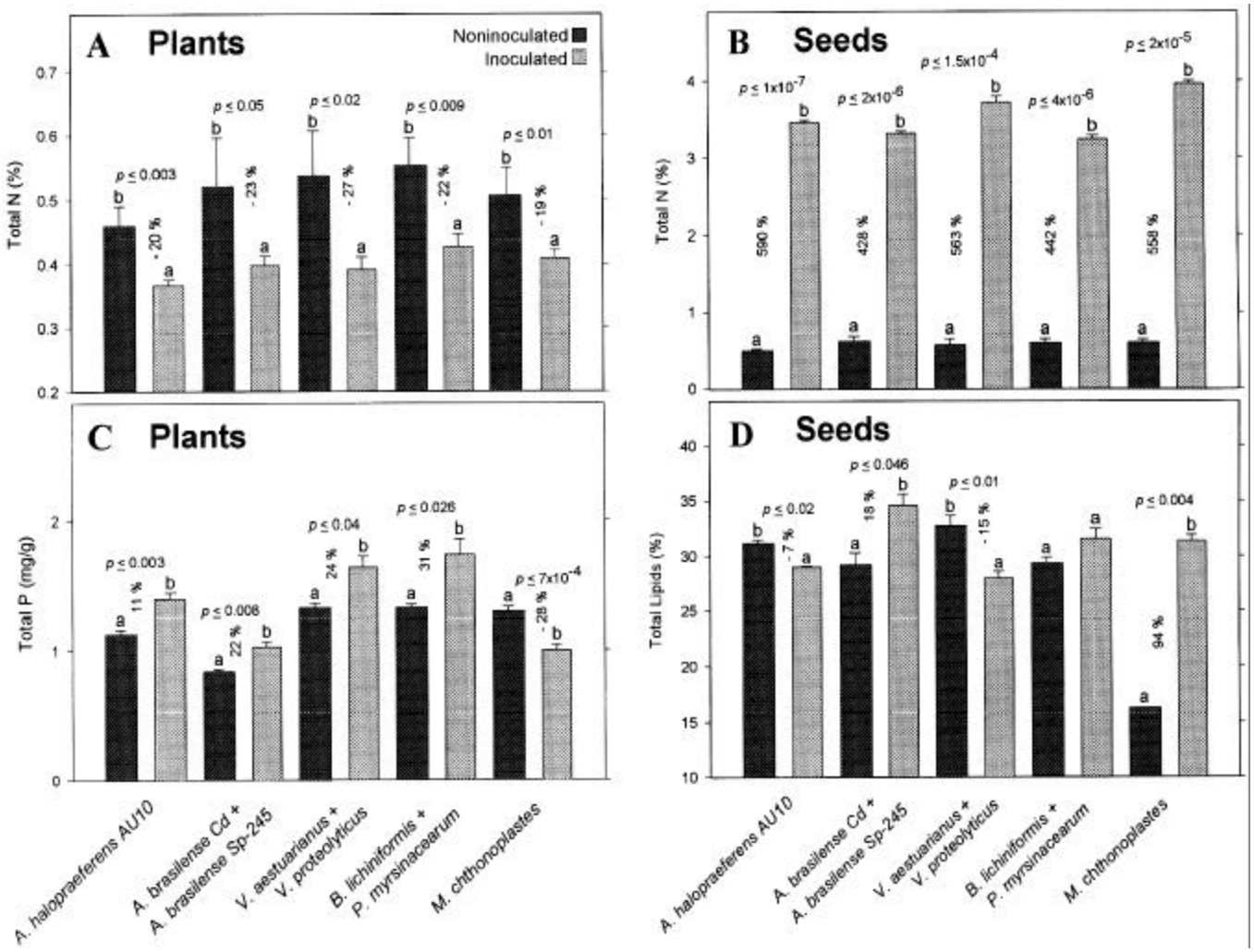

Fig.3A-D Effect of inoculation with several plant-growth-promoting bacteria on the chemical composition of *Salicornia bigelo*vii foliage and seeds at the end of the growing season. **A** Total N content of foliage, **B** total N content of seeds, **C** total P content of foliage, **D** total lipid content of seeds. *Pairs of values* (inoculated and uninoculated treatments) that differed significantly at the P level denoted above the pair, are indicated by *different lower case letters.* Vertical numbers above each pair indicate a significant increase (or decrease; %) in inoculated plants as compared to uninoculated plants. *Bars* represent SE. *Absence of a bar* in any column indicates negligible SE

with seawater, inland saline deserts could be irrigated with saline lake water, and arid zones could be irrigated with brackish water, seawater agriculture has not progressed much beyond the prototype stage developed decades ago (Glenn et al. 1998).

Because none of the top five plant species consumed by man can tolerate high levels of salt (NaCI, the most abundant moiety in seawater, is also the most destructive for plant development), seawater agriculture would only be feasible if salt-tolerant plants that yield useful products at levels high enough to justify the expense of pumping and transporting water from the sea could be cultivated. Our working hypothesis was based on the assumption that, instead of breeding a common, high yield crop for salt tolerance, it may be easier to select and domesticate wild plants that already have high salt tolerance and possess desirable crop characteristics. Inoculation with PGPB may help to improve crop yields.

From over 2000 species of known halophytes, some of which produce as much dry matter as irrigated alfalfa, *S. bigelovii* was selected as the most promising crop species (Glenn et al. 1991, 1998). *S. bigelovii,* as for many other halophytes, has been cultivated on only a marginal scale and mainly in developing countries. As a terrestrial plant growing in the intertidal zone, *S. bigelovii* reacts towards nutrients as many other common crop plants do. It responds to N fertilization (Covin and Zedler 1988) and to sewage irrigation (Rodriguez-Medina et al. 1998). It was therefore reasoned that it might also respond to PGPB, some of which, like *Azospirillum* sp., facilitate nutrient acquisition by plants (Bashan and Holguin 1997).

Using potential PGPB, isolated from the same environment in which wild *Salicornia* plants were grown (mangrove ecosystems) and two known halotolerant species of *Azospirillum,* we were able to demonstrate the feasibility of using contemporary agricultural technology in a program to domesticate a wild plant. This wild plant responded to *Azospirillum* spp. inoculation as many crops do, by increasing

Table 2 Fatty acid analyses of *S. bigelovii* seeds. *Numbers in rows* followed by a *different letter* differ significantly according to Student's *t*-test. *NS* No significant difference

Treatment	Fatty acid	Inoculated (%)	Noninoculated (%)	Significance level (*P*)
A. halopraeferens	Palmitic	24.76 b	11.43 a	7×10^{-6}
A. halopraeferens	Linoleic	75.24 a	88.57 b	0.003
A. brasilense Cd plus Sp-245	Palmitic	13.67 a	13.88 a	NS
A. brasilense Cd plus Sp-245	Linoleic	86.33 a	86.12 a	NS
V. aestuarianus plus *V. proteolyticus*	Palmitic	25.13 b	13.66 a	0.004
V. aestuarianus plus *V. proteolyticus*	Linoleic	74.87 a	86.34 b	0.01
Phyllobacterium sp. plus *B. licheniformis*	Palmitic	23.48 b	11.35 a	0.0002
Phyllobacterium sp. plus *B. licheniformis*	Linoleic	76.52 a	88.65 b	0.0002
M. chthonoplastes	Palmitic	16.86 a	12.48 a	NS
M. chthonoplastes	Linoleic	83.14 a	87.52 a	NS

size, dry weight, and several plant growth variables. It also responded positively to other marine PGPBs. As far as we know, this is one of the few reported cases of inoculation of wild plants with *Azospirillum* (Puente and Bashan 1993; Zaady et al. 1993; Puente et al. 1999) and the first of marine grasses.

Salicornia sp. has good potential as both a forage (Swingle et al. 1996) and an oilseed crop. Under natural growth conditions, the seeds contain about 30% oil (particularly linoleic acid) and about 35% protein (similar to soybean and other oilseed crops). The oil is polyunsaturated and similar to sunflower oil in its fatty acid composition. The expected yield is 1700 kg plant biomass 1000 m^{-2} land and 200 kg seeds 1000 m^{-2} land, a seed yield similar to that of soybean and sunflower (Glenn et al. 1998). In this study, inoculation with various PGPBs improved both characteristics; the plants were significantly larger and the seed N, protein, and P contents significantly higher. These increases may have been caused by N fixation, PO_4^{3-}-solubilization, and hormone production by the PGPBs used in this study. We do note that the larger inoculated plants had a lower N content than the uninoculated plants. This could occur if nitrogenous compounds were translocated from the plants to the seeds as the seeds matured. It is apparent that in inoculated *Salicornia* plants seeds act as a better sink for N than in uninoculated plants.

The lipid content of seeds increased only in the two least successful inoculation treatments, when plants were inoculated with a mixture of *A. brasilense* strains and with a mangrove cyanobacterium. Although these inoculations did not affect plant growth, and therefore did not improve the plant's use as a forage crop, they did improve the nutritive value of the seeds. This study may suggest that consideration be given to the end use of the plant in determining which inoculant to use.

Inoculation of plants with mixtures of PGPBs is a promising future avenue for inoculation technology (for a review see Bashan and Holguin 1997). In this study, the best inoculum for promoting plant growth contained a mixture of marine PO_4^{3-}-solubilizing bacteria and N_2-fixing bacteria. However, it should also be noted that a mo noculture of *A. halopraeferens,* a little known halotolerant *Azospirillum* species isolated from Kallar grass growing in salt marshes in Pakistan (Reinhold et al. 1987), also impacted significantly on several plant growth variables by an as yet unknown mechanism.

Azospirillum species are known to affect plant growth during the early stages of plant development (Levanony and Bashan 1989; Jacoud et al. 1999), with the most marked effects occurring within days or a few weeks after inoculation of young plants (Crews et al. 1996). In our experiment, enhanced growth of *Salicornia* plants was not apparent during the first 3 months of growth. This may indicate halophytes respond differently to plant inoculation than do common terrestrial plants.

Although the plants in this study were not cultivated in a flooded environment, *Salicornia*'s natural habitat, mainly to prevent cross contamination of control plots in an aquatic environment by motile PGPB, an effort was made to mimic, even in part, the natural growth conditions of *Salicornia*. The potting substrate had a high organic matter content similar to that found for *Salicornia* plants growing in mangrove swamps, the pots were flooded daily with seawater as occurs in the tidal zone, and the net house was located close to a wild *Salicornia* population where it was protected against herbivores and birds and provided a means of preventing bacterial cross contamination.

In sum, this study demonstrates the feasibility of using bacterial inoculants to promote the growth of halotolerant forage and oilseed crops in proposed seawater irrigated agriculture.

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References

Ayala F, O'Leary JW (1995) Growth and physiology of *Salicornia bigelovii* Torr. at suboptimal salinity. Int J Plant Sci 156:197-205

Bashan Y (1986a) Significance of timing and level of inoculation with rhizosphere bacteria on wheat plants. Soil Biol Biochem 18:297-301

Bashan Y (1986b) Alginate beads as synthetic inoculant carriers for the slow release of bacteria that affect plant growth. Appl Environ Microbiol 51 :1089-1098

Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiological advances (1990-1996). Can J Microbiol 43 :103-121

Bashan Y, Holguin G, Lifshitz R (1993) Isolation and characterization of plant growth-promoting rhizobacteria. In: Glick BR, Thompson JE (eds) Methods in plant molecular biology and biotechnology. CRC, Boca Raton, Fla., pp 331-345

Bashan Y, Puente ME, Myrold DD, Toledo G (1998) In vitro transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria to black mangrove seedlings. FEMS Microbiol Ecol 26:165-170

Benito 1, Onaindia M (1991) Biomass and aboveground production of four angiosperms in Cantabrian (N. Spain) salt marshes. Vegetatio 96:165-175

Bligh EG, Dyer WL (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Boyko H (1967) Salt-water agriculture. Sci Am 216:89-96

Covin JD, Zedler JB (1988) Nitrogen effects on *Spartina foliosa* and *Salicornia virginica* in the salt marsh at Tijuana Estuary, California. Wetlands 8:51-65

Creus CM, Sueldo RJ, Barassi CA (1996) *Azospirillum* inoculation in pregerminating wheat seeds. Can J Microbiol 42:83-86

Diab A, A1 Gounaim MY (1984) Distribution of *Azotobacter,* actinomycetes, cellulose-degrading, acid-producing, and phosphate-dissolving bacteria in desert and salt marsh soils of Kuwait. Zentralbl Mikrobiol 139:425-433

Ellison AM (1987) Density-dependent dynamics of *Salicornia europaea* monocultures. Ecology 68:737-741

Glenn EP, O'Leary JW, Watson MC, Thompson TL, Kuehl RO (1991) *Salicornia bigelovii* Torn*:* an oilseed halophyte for seawater irrigation. Science 251 :1065-1067

Glenn EP, Brown JJ, O'Leary JW (1998) Irrigating crops with seawater. Sci Am 279:76-81

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895-914

Hartmann A, Zimmer W (1994) Physiology of *Azospirillum.* In: Okon Y (ed) *Azospirillum*/plant associations. CRC, Boca Raton, Fla., pp 15-39

Holguin G (1998) Technical report. Consejo Nacional de Ciencia y Tecnologia, Mexico City

Holguin G, Bashan Y (1996) Nitrogen-fixation by *Azospirillum brasilense* Cd is promoted when co-cultured with a mangrove rhizosphere bacterium *(Staphylococcus* sp.). Soil Biol Biochem 28:1651-1660

Holguin G, Guzman MA, Bashan Y (1992) Two new nitrogenfixing bacteria from the rhizosphere of mangrove trees, isolation, identification and in vitro interaction with rhizosphere *Staphylococcus* sp. FEMS Microbiol Ecol 101:207-216

Jacoud C, Job D, Wadoux P, Bally R (1999) Initiation of root growth stimulation by *Azospirillum lipoferum* CTRL during maize seed germination. Can J Microbiol 45:339-342

Jefferies RL, Davy AJ, Rudmik T (1981) Population biology of the salt marsh annual *Salicornia europaea* Agg. J Ecol 69:17-31

Kitson RE, Mellon MG (1944) Colorimetric determination of phosphorus as molybdivanado-phosphoric acid. Ind Eng Chem Anal 16:379-383

Levanony H, Bashan Y (1989) Enhancement of cell division in wheat root tips and growth of root elongation zone induced by *Azospirillum brasilense* Cd. Can J Bet 67:2213-2216.

Ogoshi A, Kobayashi K, Homma Y, Kodama F, Kondo N, Akino S (eds) Plant growth-promoting rhizobacteria - present status and future prospects. Faculty of Agriculture, Hokkaido University, Sapporo, Japan

Puente ME, Bashan Y (1993) Effect of inoculation with *Azospirillum brasilense* strains on the germination and seedlings growth of the giant columnar Cardon cactus *(Pachycereus pringlei).* Symbiosis 15:49-60

Puente ME, Holguin G, Glick BR, Bashan Y (1999) Root-surface colonization of black mangrove seedlings by *Azospirillum halopraeferens* and *Azospirillum brasilense* in seawater. FEMS Microbiol Ecol 29:283-292

Reinhold B, Hurek T, Fendrik I, Pot B, Gillis M, Kersters K, Thielemans S, De Ley J (1987) *Azospirillum halopraeference* sp. nov., a nitrogen-fixing organism associated with roots of Kallar grass *(Leptochloa fusca* (L.) Kunth). Int J Syst Bacteriol 37:43-51

Rey JR, Shaffer J, Crossman R, Tremain D (1990) Above-ground primary production in impounded, ditched, and natural Batis *Salicornia* marshes along the Indian River Lagoon, Florida, U.S.A. Wetlands 10:151-171

Rodriguez-Medina M, Lanza-Espino G de la, Hernandez-Pulido S (1998) Decomposition of *Salicornia subterminalis* in Caimanero Lagoon, Mexico. Cienc Mar 24:241 251

Swingle RS, Glenn EP, Squires V (1996) Growth performance of lambs fed mixed diets containing halophyte ingredients. Anim Feed Sci Technol 63:137-148

Toledo G, Bashan Y, Soeldner A (1995) In vitro colonization and increase in nitrogen fixation of seedling roots of black mangrove inoculated by a filamentous cyanobacteria. Can J Microbiol 41:1012-1020

Troyo-Dieguez E, Breceda Solis-Camara AM (1992) Germina cion de *Salicornia bigelovii* Torr. (Chenopodiaceae) bajo di ferentes concentraciones de agua marina. Southwest Nat 37:22 27

Troyo-Dieguez E, Ortega-Rubio A, Maya Y, Leon JL (1994) The effect of environmental conditions on the growth and development of the oilseed halophyte *Salicornia bigelovii* Torr. in arid Baja California Sur, Mexico. J Arid Environ 28:207-213

Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol Fertil Soils 30:460-468

Zaady E, Okon Y, Perevolotsky A (1993a) Growth promo tion of Mediterranean herbaceous swards to inoculation with *Azospirillum brasilense.* J Range Manage 47:12-15