Biol Fertil Soils (2009) 45:585–594
DOI 10.1007/s00374-009-0367-x

ORIGINAL PAPER

Enhanced establishment and growth of giant cardon cactus in an eroded field in the Sonoran Desert using native legume trees as nurse plants aided by plant growth-promoting microorganisms and compost

Yoav Bashan · Bernardo Salazar · Ma. Esther Puente · Macario Bacilio · Robert Linderman

Received: 10 December 2008 / Revised: 25 February 2009 / Accepted: 27 February 2009 / Published online: 13 March 2009

Abstract To evaluate the feasibility of long-term desert reforestation technology of mixed vegetation, cardon cactus (*Pachycereus pringlei*) seedlings from indoor and outdoor nurseries were planted in the field adjacent to one seedling of potential legume nurse trees: mesquite amargo (*Prosopis articulata*), yellow palo verde (*Parkinsonia microphylla*), and blue palo verde (*Parkinsonia florida*). Some of the planting holes were also supplemented with common dairy compost. Additionally, the combinations of legume tree–cactus were inoculated with either a consortium of desert arbuscular mycorrhizal (AM) fungi, plant growth promoting bacteria (PGPB; the diazotroph *Azospirillum brasilense* Cd, and the phosphate solubilizer *Paenibacillus* sp.), or a mixture of all. The field experiments were evaluated periodically during 30 months for survival and growth. Cardons reared in an outdoor screen house survived better in the field than those reared in a controlled growth chamber and hardened later outdoors. Association with any legume nurse tree increased survival and enhanced growth of untreated cardons. For cardons growing alone, application of either compost, AM fungi, and all the treatments combined increased survival. For these plants, no treatment affected plant growth during the first 3 months after transplanting. Later, all treatments, except for AM fungi, enhanced plant growth. However, only 2 years after transplanting the enhanced growth effect of AM fungi was also significant. In the presence of the legume nurse trees, transient positive effects on cardon growth were recorded. General evaluation after 30 months of cultivation showed that the treatments positively affected cardon growth when growing alone or in combination only with mesquite amargo but not with the other two legume trees. This study proposes that young legume trees have the capacity to enhance survival and growth of cardon cactus, depending on the legume cactus combination. Additional treatments such as compost or PGPB can either amplify the effect or else attenuate it.

Keywords *Azospirillum* · Cardon cactus · Compost · Desert · Mesquite · Mycorrhizae · Nurse plants · *Pachycereus* · Palo verde · *Parkinsonia* · Plant growth-promoting bacteria · PGPB · PGPR · *Prosopis* · Reforestation · Soil erosion

Y. Bashan (✉) · B. Salazar · M. E. Puente · M. Bacilio
Environmental Microbiology Group,
Northwestern Center for Biological Research (CIBNOR),
Mar Bermejo 195, Colonia Playa Palo de Santa Rita,
La Paz, Baja California Sur 23090, Mexico
e-mail: bashan@cals.arizona.edu

Y. Bashan
Department of Soil, Water and Environmental Science,
The University of Arizona,
Tucson, AZ, USA

R. Linderman
USDA-Agricultural Research Service,
Corvallis, OR, USA

Present Address:
R. Linderman
Plant Health, LLC,
5480 NW Belhaven Drive,
Corvallis, OR 97330, USA

Introduction

Reforestation of eroded lands undergoing erosion processes in the Sonoran Desert in northwest Mexico and southwest USA (Grover and Musick 1990) involves at least three stages. First, it is necessary to establish fast cover with native annuals to arrest the erosion process, a task difficult to achieve when the rainfall is very low (Banerjee et al. 2006; Roundy et al. 2001). Second is the establishment of cover with shrub-like plants for short- and medium-term reforestation (Bean et al. 2004). Last is the establishment of a long-term soil erosion prevention program using long-lived and slow-growing tree-shaped cacti like the giant cardon cactus (*Pachycereus pringlei*; Bashan et al. 1999, 2000b), the world's most massive cactus. Cardons normally develop to heights of 5–18 m tall and stabilize the soil at a radius of over 30 m around the plant.

In the southern Sonoran Desert in Baja California, Mexico, cardon seedlings commonly occur under the canopy of mature (20 years or older) mesquite trees (Carrillo-Garcia et al. 1999) and less commonly with other legume trees or shrubs. The cactus seedlings concentrate under the canopy of the mesquite in a resource island (Garner and Steinberger 1989) where they have better nutrition, shade, more water, clays, and organic matter, and abundant arbuscular mycorrhizal (AM) fungi (Arriaga et al. 1993; Bashan et al. 2000a; Carrillo-Garcia et al. 2000a; Valiente-Banuet and Ezcurra 1991). Young cardon seedlings are seldom associated with young legume trees. Although the association between the mature mesquite tree and the cactus seedlings is positive (Bashan et al. 2000a; Carrillo-Garcia et al. 1999, 2000a, b), its main drawback is the need for mature mesquite. A reforestation program cannot be suspended for 20 years in order to establish mature mesquite trees required for the natural association.

The hypotheses of this study were (a) the natural succession process can be shortened by the establishment of the cardon–legume tree association when both plants are young, (b) treatments with compost and/or growth-promoting microorganisms may support the legume tree and shorten the cardon establishment period, and (c) cardons reared in conditions simulating outdoor conditions are better for reforestation than those reared under controlled conditions and hardened later. To test these hypotheses, legume–cardon combinations were created and tested in the field. We used three legume trees common to the southern Sonoran Desert to evaluate which species was best for this association. All combinations would be tested with two types of cardon seedlings, grown indoors or outdoors, and treated with growth-promoting microorganisms and compost.

Materials and methods

Organisms

Plant species used were the legume trees, mesquite amargo (*Prosopis articulata* (S. Watson)), yellow palo verde or foothill palo verde (*Parkinsonia microphylla* (Torr.), blue palo verde or palo junco (*Parkinsonia florida* (Benth. Ex A. Gray) S. Wats), and the giant cardon cactus (*Pachycereus pringlei* (S. Wats Britt. & Ross). Microorganisms used were the plant growth-promoting bacteria (PGPB) *Azospirillum brasilense* Cd (Bashan et al. 2004) and the phosphate-solubilizing bacteria *Paenibacillus* sp. strain RIZO1 (EF123224, GenBank of NCBI, National Center for Biotechnology Information, USA; Puente et al. 2004a). The AM fungi used were propagated in pot cultures as a consortium of mostly *Glomus* sp. and several unidentified native species found in resource islands (Halvorson et al. 1994) under mesquite trees in the southern Sonoran Desert (Bashan et al. 2000a, b; Carrillo-Garcia et al. 1999).

Microbial cultivation

Bacteria *A. brasilense* Cd and *Paenibacillus* sp. strains were cultivated on tryptone–yeast extract–glucose medium supplemented with microelements, as described before, for 24 h at 30°C on a shaker at 120 rpm (Bashan et al. 2002a, b). The two bacterial species were formulated into dry microbead inoculants preparation made of alginate as described before (Bashan et al. 2002a, b) using specialized equipment (http://www.bashanfoundation.org/bead.html, accessed February 15, 2009).

Production of AM inoculum

AM fungal inoculum was prepared in pot cultures with sorghum plants (*Sorghum bicolor* [L.] Moench). Plants were cultivated in 10 l plastic pots containing resource island soil from the field experiment that contained high levels of AM fungi (Carrillo-Garcia et al. 1999). Plants were cultivated from seeds in a screen house under ambient conditions at light intensities approximately one half of full sunlight (1,000 μmol m^{-2} s^{-1}) for 6 months and irrigated with tap water when necessary. They were fertilized periodically with 1.5% NK commercial garden fertilizer supplemented with low P content (0.1%) to enhance AM fungal colonization of roots. The plants were senescent at harvest. At harvest, roots were analyzed for the level of AM colonization using the line intersect method (Marsh 1971; Vierheilig et al. 1998). The level of root colonization was 54.5%. This method was also used to evaluate AM colonization 2 years after inoculation.

Upon excising the stems, the rooted soil clumps were air-dried for 2 weeks before being broken down for spore counts. Spore numbers were determined by wet-sieving (45-, 75-, 100-, and 200-μm sieve openings), decanting, and sucrose-gradient centrifugation (Brundrett et al. 1994) of the soil samples. The AM fungal spore count of the pot culture was 308 spores per 100 g of soil.

Compost

The common dairy–wheat compost used was produced for cultivation of cardon cactus and its composition was described earlier (Bacilio et al. 2006). It was applied only in the planting hole at the rate of 1:8 (compost/soil, 735 g:10 kg, *v/v*).

Application of inoculants

Bacterial inoculant made of alginate microbeads was attached to the seeds of wild trees as described for wheat plants (Bashan et al. 2002a, b) at a level of 1.2×10^{6}cfu g^{-1} soil of *Paenibacillus* sp. and 1×10^{6}cfu g^{-1} soil of *A. brasilense*. After pot culturing on sorghum roots, roots with AM were extracted and cut into small pieces (≤0.5 cm length). Then, the root pieces were mixed back into the conserved soil. A mixture of soil–root (213 g) inoculant was used to inoculate each legume tree. Inoculants and compost for the field experiments were manually mixed into the local soil and maintained in 30 kg sacks at ambient temperature for about 2 weeks until used.

Collection of seeds

Seeds of mesquite amargo, yellow palo verde, and blue palo verde were collected from ten native trees (200 g plant^{-1}) located in fields surrounding the settlements of El Centenario and El Comitan, 15 km from La Paz, Baja California Sur, Mexico (24°07′36″ N, 110°25′48″ W) in July 2003. Seeds of giant cardon cactus were collected twice, in August 2001 and 2004, in El Sargento area (24°5′ N, 110°10′). Collections were made for each according to the time when seeds of these species mature and were kept in hermetically sealed boxes at ambient temperature (Puente and Bashan 1993). Only seeds that dried on the tree inside the pods were used because only these seeds achieved >80% germination. Pods visibly infected (perforated) with beetle larvae of *Acanthoscelides obtectus* (Say) were discarded. Mesquite seeds were extracted from the pods by a mechanical mill (perforation foil, 1 cm diameter, P. Felker, personal communication). Pod debris was discarded, and seeds were again checked for the beetle larvae (visible as a black dot on the upper third of the seeds). Healthy seeds were stored at 2–6°C for several months. Seeds of the two palo verde species were extracted manually. All seeds were mature (brown color) except for seeds of blue palo verde that germinated better when they were half green. Seeds of cardon were extracted after burning the spikes of the fruits and washing the pulp (Puente and Bashan 1993).

Greenhouse plant cultivation

Some 1,550 legume seedlings to be used for transplanting to the field experiments were cultivated in 30×10 cm pots (150 pots for each of the legume trees), each containing 1.5 kg of nonsterile eroded desert soil. Some 1,100 cardon cactus seedlings were cultivated in growing trays, each with 100 3×3×7-cm mini-individual pots. Legume trees were cultivated as follows: Black plastic commercial sleeves (30×10 cm in diameter) with drainage were used, each containing 1.5 kg of eroded desert soil collected from the field experiment site. The soil was sieved to 1 mm (Tyler equivalent 16 mesh No.18 USA Standard Testing Sieve). Seeds were washed with 2% Tween 20 (polyoxyethylene–sorbitan monolaurate; Sigma) under constant agitation for 5 min, thoroughly washed with tap water, disinfected in 1% (the two palo verde species) and 3% (mesquite) commercial NaOCl under constant agitation for 5 min, thoroughly washed with sterile tap water, and soaked in water in a steel strainer at 55°C for 2 min (mesquite) and boiling for 1 min for the two species of palo verde (Scott 2006). Seeds were germinated in large Petri dishes with water-wet sterile filter paper towels about 1 cm apart and incubated in the dark at 33°C in a growth chamber (Conviron, Model 125 L, Manitoba, Canada). Defective seedlings were discarded. Apparently healthy seedlings were transferred to large test tubes (12.5 cm long, 1.5 cm in diameter) containing 10 ml of soil after 14 days or until they reached about 10 cm tall. Seedlings of similar size were planted in pots.

Each pot contained initially five seedlings. After 1 month, the pots were thinned to one plant per pot of similar size seedlings of naturally variable native plants. All plants were grown in a screen house on elevated metal net beds at ambient temperature (15–35°C, n/d) irrigated with tap water every 2 days (small plants) and later every 3 days. Fertilization (commercial 0.5% NPK 18:18:18) was given after 10 days, 2 and 5 months to all species of legume trees. Cardon cacti were cultivated without fertilization under two sets of conditions: (a) in a screen house at the Centro de Investigaciones Biológicas del Noroeste experiment station under southern Sonoran Desert outdoor environmental conditions for 9 months (Carrillo-Garcia et al. 1999) or (b) in a growth chamber for 2 years. The seeds were first disinfected (2% Tween-20 [Sigma] for 10 min, rinsed with sterile water, 3% commercial NaOCl for 5 min, thoroughly washed in sterile tap water for 10 min). Then, the seeds were sown into and grown in trays as described above. They were irrigated with tap water once a week with marginal amount of water as required by these slow growing cacti (Bacilio et al. 2006). The second group of cardons was grown in nonsterile soil in a walk-in growth chamber (35°C, 45% relative humidity, 200 μmol photon m^{-2} s^{-1} photoperiod 12:12 hours light/dark ratio for 2 years). These plants (about 5 cm tall on average) were then gradually hardened in the outdoor screen house for an additional 6 months by increasing light intensities using five shading screens of variable shading levels (from 300–

1500 μmol photons m^{-1} s^{-1}) to allow them to adapt to outdoor conditions.

Planting hole design in the field

The planting hole was designed to maximize contact between inoculants and plants, conserve the limited amount of water, and reduce risk of predation by potential herbivores (that might pass the fence). Each tree was transplanted into identical holes (25 cm deep, 40 cm in diameter) excavated by a commercial garden excavator. About 80% of the soil removed from the hole was mixed with the bacterial and AM fungal inoculants and the compost (as described earlier) and returned to the hole. Each planting hole received 6 kg of this inoculated soil at the day of planting (total 180 kg per treatment, 900 kg per experiment).

Two seedlings were planted 20 cm apart in each hole and the soil was lightly packed at a level about 5 cm below the surrounding area, thereby creating a sunken planting site. A layer of sterilized (105°C–24 h) buffel grass (*Cenchrus cillaris* L.) was placed on the packed soil in a 5-cm-thick layer to reduce water evaporation and provide camouflage against herbivores. In treatments where only cardon cactus seedlings were involved, they were planted in the center of the hole.

Field cultivation

Protection against herbivores

The area of the field experiments was located in a natural reserve having natural populations of herbivores such as Merriam's kangaroo rat (*Dipodomys merriami*), jackrabbit (*Lepus californicus xanti*), rabbit (*Sylvilagus bachmani peninsularis*), and the Spiny-tailed Iguana (*Ctenosaura hemilopha*). All previous field experiments in this area were consumed by these herbivores (J.L. Leon de la Luz, personal communication). To avoid predation of the transferred plants, the entire area of the seven experiments was protected by three forms of security: (a) enclosure by a metal stainless chicken net (1 cm mesh) buried 30 cm below ground (to avoid borrowing underneath) and 65 cm above ground supported by T-shape poles. On the top of the metal net, a 30-cm-wide plastic sheet, equipped with large holes to reduce wind resistance, was mounted to prevent the rodents from climbing up the net into the closed area. All animals (rattle snakes, lizards, iguanas, and rodents) trapped in the enclosed metal net were captured and released to the nearby reserve. Additionally, a search for local underground inhabiting rodents was done systematically on the entire area for about 2 weeks until the last one was removed. (b) Antirodent commercial venom (not toxic to birds) was spread at the day of planting. (c) The plating holes were camouflaged with sterilized buffel grass cut to small pieces of about 10 cm that completely covered the soil around the plants. It was assumed that the small trees would be camouflaged against occasional herbivore by the most common weed in the area. Thus, the transplants (in a carpet of buffel grass) were less attractive to any herbivores that entered the enclosure (Alvarez Castañeda S. T., personal communication). The invasive grass was previously oven-sterilized over night at 80°C to kill its seeds. The thick grass layer also functioned as mulching to reduce water evaporation from the soil. The achieved security efficiency was 100% because not a single predation of trees occurred during the 3 years of the experiments.

Irrigation

Upon planting, the holes were filled with tap water until the entire hole was soaked. These experiments were devised initially to use only rainfall for the duration of 3 years. In this area, the multiyear average is 180 mm and in a good rainy year, rainfall might reach 300 mm, mainly by short episode rain storms (hurricanes). In these “wet” periods, desert perennial vegetation is normally established (Drezner 2006). Unfortunately, the experiments were conducted in drought years (common in the Baja California peninsula), and the rainfall was 177 (2004), 58 (2005), and 73 mm (2006; Comisión Nacional de Aguas, Mexico). Consequently, the amount of water that the plants received was adjusted, by irrigation, to 300 mm of rain with various amount and frequencies depending on the natural precipitation on each month. In the first 2 years, these irrigations were done by irrigating every planting hole manually with 1 l of tap water per irrigation. In the third year, when the planting holes had almost disappeared, the entire area was surface irrigated by a water tanker, when necessary, with the equivalent of 10–20 mm of rain. The total amount of water that the trees received in any given year, naturally or by irrigation, did not exceed 300 mm of rain per year.

Maintenance of the field

No natural diseases or pests appeared in the field experiments for 3 years. Only several mesquite trees were infested in the first year by red spider (*Tetranychus urticae)* nets. Those were treated locally by two liquid detergent applications. Because the surrounding area is completely covered by invasive buffel grass, the experimental area was weeded twice a year after major rains to avoid the common invasion of weed species to abandoned desert farmlands in the Sonoran Desert.

Experimental field design and statistical analysis

The experiments using only cardon cactus were adjacent to the field experiments with the legume trees having the same treatment scheme. The following treatments were applied for each experiment: (1) control cacti growing in eroded soil, (2) cacti inoculated with AM fungi, (3) cacti inoculated with a mixture of two PGPB, (4) cacti growing in soil amended with compost, and (5) cacti growing in soil amended with compost and inoculated with AM fungi and PGPB. Treatment selection was based on a previous screen house study evaluating the potential benefits of these treatments (Bashan et al. unpublished).

All experiments had a randomized block design in five blocks each. Each bock contained the five treatments, and their location within the block was chosen by a computer program for random numbers.

Experiments with cardon alone (indoor and outdoor source of seedlings) Each treatment contained 30 cactus seedlings for a total 150 cacti per experiment. The holes were dug in W-shape configuration to minimize competition and provide the same volume of soil per plant. Because cardons are small plants in the initial years of growth (Carrillo-Garcia et al. 2000a), the planting distance was 0.5 m between plants and the size of the plots was 2×1 m for each treatment and 2×5 m for each block.

Experiments involving two tree species in dense configuration The design and the treatments were the same as described for the cardon-only experiments above. The difference was in the density of the two trees in each planting hole (one cardon cactus and one legume tree). Each treatment had 25 cardons + 25 legume tree seedlings for a total of 250 plants. Planting distance between holes was 0.5 m in W-shape configuration. As above, the size of each treatment was 2×1 m and the size of each block was 2×5 m. In some cases where the associated legume tree did not survive but the cardon did, the data for this cardon were not included in the analysis.

General analysis of plant growth In addition to analysis of the effect on survival and plant growth of individual treatments, a general analysis of plant development was done. This was accomplished by (a) combining all of the growth data of all the surviving cardons after 30 months from all external treatments (inoculation with AM fungi, PGPB, and compost amendment) and (b) combining the data of all cardons growing with each of the legume tree species without additional treatment.

After normalization of the data, results of all experiments were analyzed by one-way analysis of variance (ANOVA) and then by Tukey's HSD post hoc analysis at the significance level of $P \leq 0.05$. Data in percentage were converted to arcsin before analysis, and statistical software was used (Statsoft™, Tulsa, OK, USA).

Results

Survival of two nursery-type cardon cactus in the field growing with or without a nurse legume tree after 30 months

In general, screen-house reared cardons survived better than growth-chamber reared cardons, regardless of the hardening procedure used for the latter cardons, the older age of the latter seedlings, or the presence of a nurse plant (Fig. 1a–d, capital letter analysis). In screen-house reared plants, survival percentage increased in the presence of any of the three nurse legume tree species (Fig. 1a–d, nontreated controls). In growth-chamber reared plants, none of the nurse plants had any effect on survival (Fig. 1a–d, nontreated controls, italics letter analysis). Addition of compost alone significantly increased survival (Fig. 1a, lower case analysis); more plants survived in this treatment than in the presence of mesquite and yellow palo verde nurse legume trees plus compost (Fig. 1a–d, compost treatment, italics letter analysis). AM inoculum combined with a nurse tree did not improve the survival of screen-house reared cardons (Fig. 1a–d, italics letter analysis). Inoculation with PGPB had no effect on survival of cardons in the presence of any nurse tree. PGPB enhanced survival of only growth-chamber reared cardons growing alone (Fig. 1a–d, italics letter analysis). The combined treatment increased survival of only screen-house reared cardons growing alone or with mesquite (Fig. 1a, b, lower case letter analysis) compared to nontreated controls.

For screen-house reared cardons growing alone, amendment of soil with compost, AM fungi, and all the treatments combined increased survival (Fig. 1a, lower case letter analysis), while for growth-chamber reared cardons, only compost and inoculation with PGPB improved survival (Fig. 1a, lower case letter analysis). However, when a nurse legume tree was growing with the cactus, regardless of the origin of the cardon seedlings, most of the treatments had no effect on cardon survival (Fig. 1b–d, lower case letter analysis). Only in four instances (out of 24 combinations of treatments) was survival enhanced to a moderate, but statistically significant, extent, three of which were caused by AM fungi.

Survival, but not growth rate, of the nurse legume trees was recorded for all the treatments combined. After 30 months of cocultivation, 100% of the mesquite amargo trees survived; survival for yellow palo verde was 98% and 95% for blue palo verde

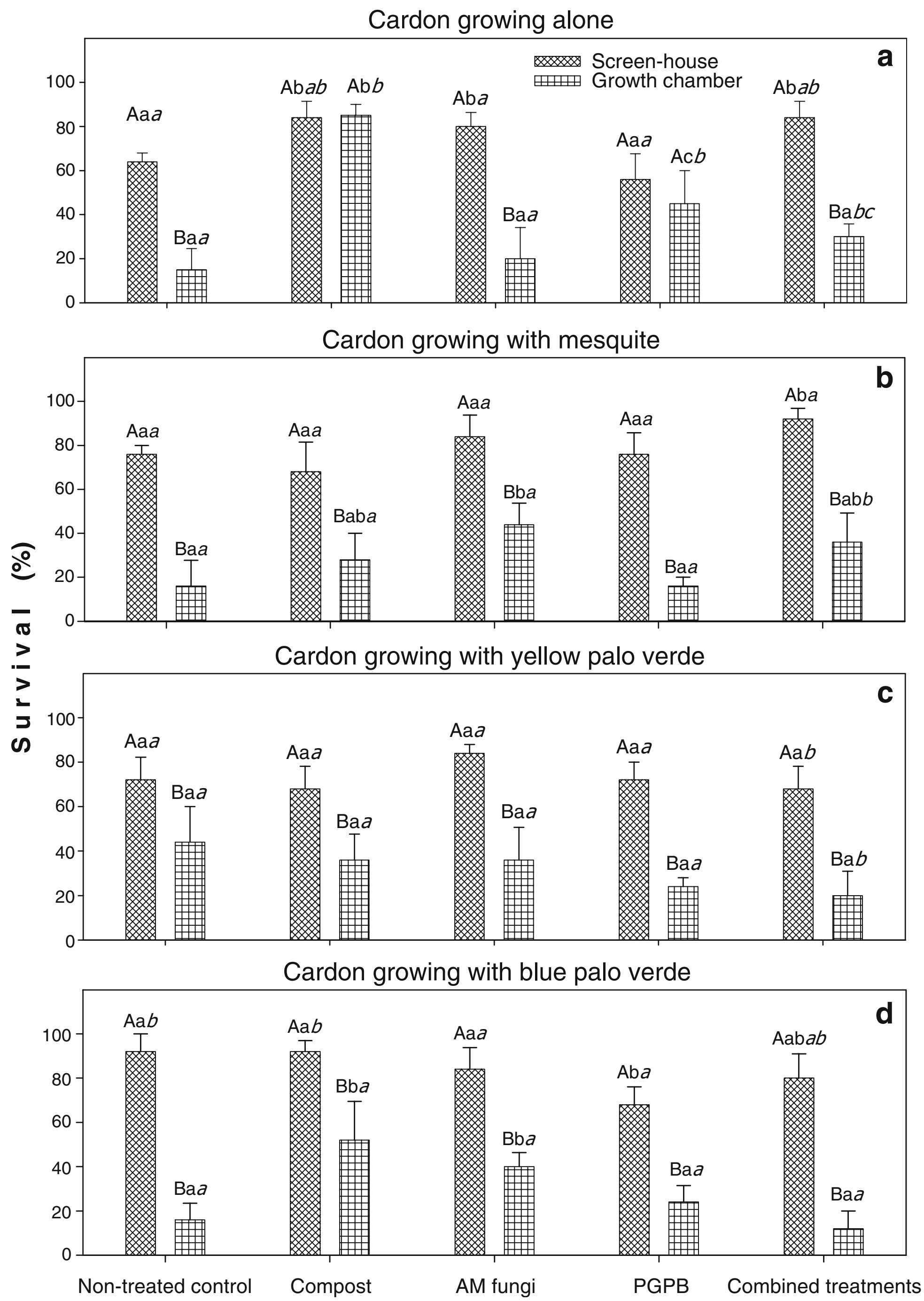

Fig. 1 Survival of cardon cacti in the field after 30 months of cultivation with and without a legume nurse tree and treated with growth-promoting microorganisms and addition of compost. *Pairs of columns* denoted by a *different capital letter* differ significantly at $P \leq 0.05$ using Student's t test (comparison between the two nurseries). *Columns in each subfigure* and for each nursery type, separately, denoted by *different lowercase letter* differ significantly at $P \leq 0.05$ using one-way ANOVA (comparison among treatments in each nursery type). Group of *four columns* related to the same plant source (screen house or growth chamber), each in a separate subfigure, denoted by *different italics lowercase letter* differ significantly at $P \leq 0.05$ using one-way ANOVA (comparison among different nurse plant species for each nursery type). *Bars* represent standard error

Growth of screen-house reared cardon cacti after 30 months in the field, with or without a nurse legume tree

Growth-chamber reared cardons survived relatively poorly and also exhibited initial size variability that later prohibited meaningful statistical analysis, compared to screen-house reared cardons. Therefore, the growth of cardons, initially of equal size, over the 30-month growth period was assessed only in screen-house reared cardons. Growth was assessed as increase in height because these young plants do not have branches.

Cardon plants grow very slowly (1–3 cm a year increase in height). During the first 3 months after transplanting of cardons grown alone, none of the treatments affected plant growth. The plants were then less than 2 cm tall (Fig. 2a). Later, all the treatments, except for AM fungi, enhanced the growth of the cardon plants. However, 2 years after transplanting the effect of AM fungi was also significant

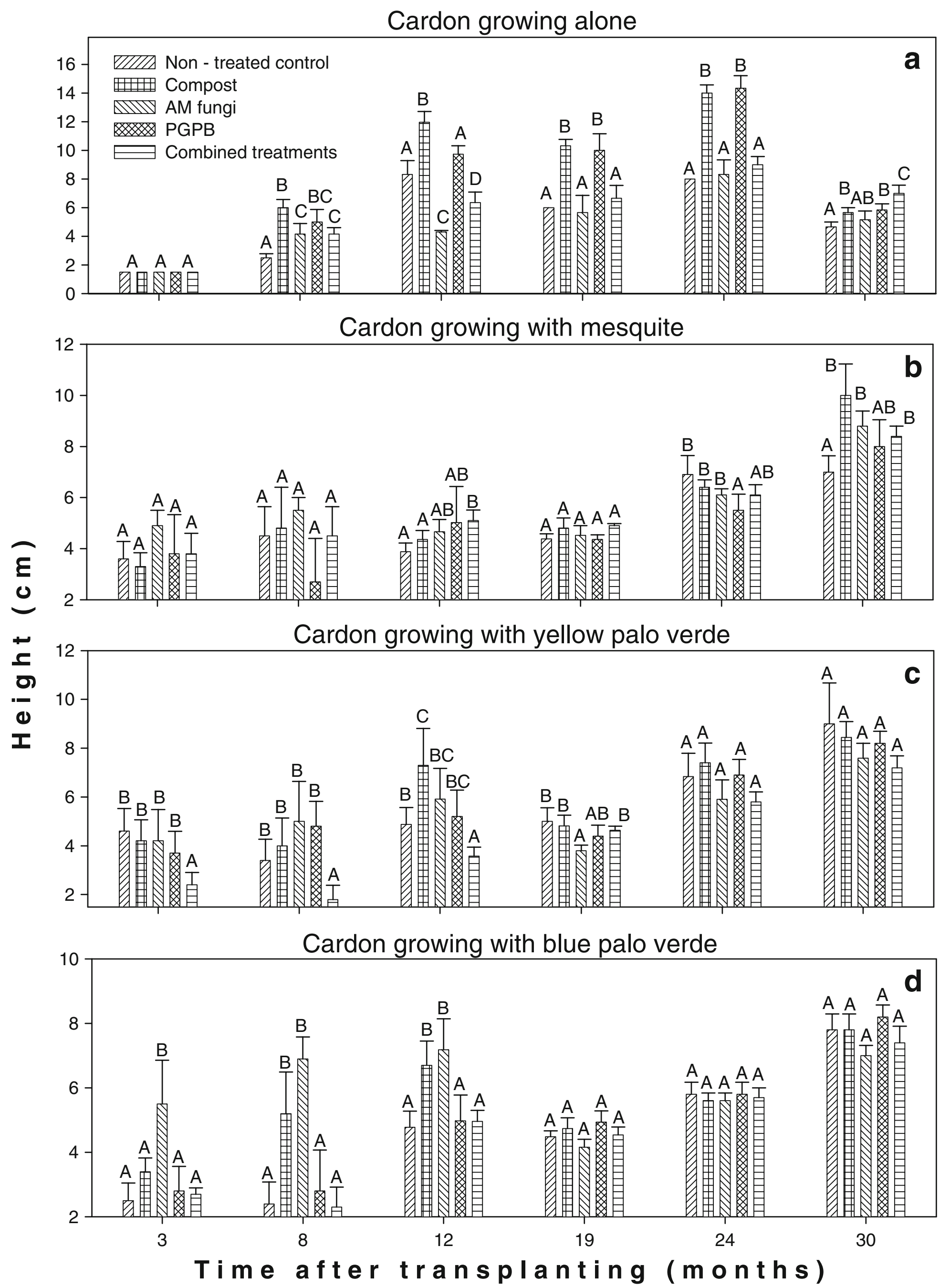

Fig. 2 Height of cardon cacti in the field for 30 months of cultivation with and without a legume tree and treated with growth-promoting microorganisms and addition of compost. *Columns within groups of five columns*, denoted by *different capital letter* differ significantly at $P \leq 0.05$ using one-way ANOVA. The seedlings were grown under screen-house cultivation. *Bars* represent standard error

(Fig. 2a). In the presence of nurse mesquite trees, there was no treatment effect for the first 2 years. Later, as cardons without treatment stopped growing, all the treatments were effective in that the cardons continued to grow (Fig. 2b). In the presence of yellow palo verde nurse trees, although a positive effect on height of cardons by compost was detected after 1 year, this effect was later diminished (Fig. 2c). In the presence of blue palo verde nurse plants, compost and AM fungi enhanced the growth of cardons during the first year, an effect that was later diminished similar to the transitory effect of compost with yellow palo verde (Fig. 2d).

General analysis of all data on the height of cardon cacti 2.5 years after transplantation showed that cocultivation of cardon with nurse legume trees, even without any additional treatment, significantly increased cardon growth. Cocultivation of cardons with mesquite combined with all the treatments increase the effect even further. However, this additional increase did not occur with the two nurse palo verde species (Table 1, capital letters analyses). When

Table 1 General analysis of the height of cardon cactus after 30 months in the field, alone, associated with a legume tree, and inoculated with plant growth-promoting microorganisms and supplemented with compost

Association	Plant height (cm)
Cardon alone	4.66±0.33 a A
Cardon + Mesquite	7.0±0.63 c B
Cardon + Yellow palo verde	9.0±1.7 cdB
Cardon + Blue palo verde	7.8±0.49 c B
Cardon alone + treatments[a]	5.92±0.39 bA
Cardon + Mesquite + treatments[a]	8.8±0.43 dC
Cardon + Yellow palo verde + treatments[a]	7.85±0.28 cB
Cardon + Blue palo verde + treatments[a]	7.6±0.26 cB

For each of the two groups (association with legume tree or association with legume + additional treatments) taken separately, numbers followed by different capital letter differ significantly at $P \leq 0.05$ using one-way ANOVA. Numbers in both graphs denoted by a different lowercase letter differ significantly at $P \leq 0.05$ using one-way ANOVA. ± standard error

[a] Each treatment (compost, inoculation with AM fungi, inoculation with two PGPB, and all the treatments combined) was applied separately, but the analysis shown is the sum effect of all treatments

all the combinations were compared in a single analysis, it was demonstrated that the treatments positively affected the growth of cardon cacti growing alone or associated with mesquite, but not when associated with the two palo verde species (Table 1, small letter analysis).

Survey of the field experiments done after 2 years of cultivation for possible AM fungi colonization of the four plant species revealed that all plants tested, including those in non-AM-treated plots and noninoculated controls were colonized by AM fungi (data not shown).

Data for each sampling time included only live plants. Although the average height of the population of cardons generally increased with time, few plants died during the long-term experiments. Dead plants were not measured and therefore, these data were not included in the calculations and presentation. This resulted in fluctuation in the data on average height of plants presented in Fig 2.

Discussion

The giant cardon cactus seldom establishes on its own on barren lands, and seedlings are always associated with resource islands developed mostly under mature mesquite trees (Carrillo-Garcia et al. 1999) or in cracks in volcanic rocks (Bashan et al. 2002b; Puente et al. 2004a). Because it is the climax plant of the Sonoran Desert and can live for several hundred years, it plays a major role in soil stabilization, prevention of soil erosion, and prevention of dust pollution. Every long-term attempt to restored human-impacted desert land should involve cardons. However, one main concern is that establishment of cacti in the field takes many years until they reach size big enough to have an impact on their surrounding soil. They are, as with most cacti, very slow growers (García-Carreño 1993; Nobel 1988, 1996). Previous greenhouse and growth chamber studies have shown that cardons responded positively in terms of survival and growth parameters to inoculation with PGPB (Bashan et al. 1999; Carrillo-Garcia et al. 2000a; Puente and Bashan 1993; Puente et al. 2004b) and to addition of compost (Bacilio et al. 2006).

Our strategy to avoid the long succession process in the desert, usually required for establishment of climax plants like cardons, was to associate its very small seedlings with relatively short-lived nurse legume trees that could aid in the cardon's establishment and growth. The latter ones grow faster, are readily colonized by AM fungi and PGPB under Sonoran Desert conditions (Bashan et al. 1999, 2000a; Carrillo-Garcia et al. 1999; Leyva and Bashan 2008; Stutz et al. 2000; Titus et al. 2003), and can prevent soil erosion from several years to several decades as a shrub until the cardons replace them as the very long-term solution (centuries).

The microbial hypothesis supporting the above strategy is that AM fungi can transfer nutrients by means of vegetative growth of hyphae from the nurse plant (legume tree) to the climax species (cardon) as has happened in agricultural crops and may serve as a means of transfer of PGPB as well. In that sense, the three legume nurse trees fulfill their purpose, as they are relatively fast growers compared to other desert shrubs (Nobel 1988). Although grasses and annual legumes can serve as a faster plant cover, the lack of sufficient rainfall in the southern Sonoran Desert and hence in the field experiment prevented their use. Although native cardons form an AM association (Carrillo-Garcia et al. 1999; Rose 1981) and a close species (*Pachycereus pecten-aboriginum*) respond positively and significantly to AM fungal inoculation (Rincón et al. 1993), giant cardon seedlings form mycorrhizal symbiosis under cultivation only after nearly 1 year after inoculation (Bashan et al. 2000a). AM mycorrhization was not evaluated in this long-term study. Alternatively, easier parameters to measure the success of treatments under field conditions were survival and height of plants. The underlying assumption was that the obtained differences were related to the applied treatments when compared to control field plots, considering the large number of trees (>2,500) that were evaluated and the rigor of the multiple statistical analyses used.

This study demonstrated that in a reforestation program, the nurse plant can be very young and does not need to be a mature tree. In nature, young associations are uncommon; only 20-year-old or older plants have nurslings (Carrillo-

Garcia et al. 1999). Additionally, this planting strategy may serve as a practical shortcut for establishment of cardon seedlings in the field. By size, in the first years, the nurse tree is much larger than the cardon nursling. Although the additional treatments of compost and growth-promoting microorganisms are essential for survival and development of cardons growing without nurse trees, the effect of the mesquite nurse tree on cardon development is more important over the long term than any microbial treatment provided at the initial growth stage. Cardons associated with the other legumes grew well without additional treatments compared to cardons growing alone. Furthermore, with time, all plants in the experiments become mycorrhizal, even those without AM fungi inoculation, due to indigenous AM fungi as is known for this area (Bashan et al. 2000a). The additional treatments had a synergistic effect only in the case of mesquite amargo, the natural most common nurse tree of cardons (Carrillo-Garcia et al. 1999) or in young age of blue palo verde that is not known as a natural nurse plant of cardon (Carrillo-Garcia et al. 1999). The association with the young nurse tree improved survival and growth in general. In specific cases, in the presence of the legumes, the cacti were somewhat smaller, probably because of space and water competition. Compost application is usually a positive supplement to soils by reducing the loss of water, improving soil structure (Neilsen et al. 1998), and supporting the early establishment and development of many plants (Stehouwer and Macneal 2003). The presence of legume nurse trees diminished the effect of the microbial treatments and especially the significant effect of addition of compost that was essential for cardon to survive in the absence of the nurse legume tree.

Commercial nurseries grow cacti as potted ornamental plants, sometimes indoors to increase productivity (http://www.cactus-mall.com/nurseryusa.html, accessed July 12, 2008). This study clearly demonstrated that if reforestation is the end goal, cardon cactus should be grown outdoors or in screen houses, because even the standard hardening process used was not sufficient to ensure survival of the majority of the population once transferred to the desert field. This may happened because solar irradiation in the southern Sonoran Desert may reach 2,500 μmol photon m^{-2} s^{-1} and therefore, a longer and more gradual hardening period may be required.

A reservation should be mentioned. These experiments were monitored for 3 years in the field. The microbial components—AM fungi and PGPB—were not monitored for this long period because the length of the effect of most PGPB on plant and especially of *Azospirillum*, used in this study, is short. The PGPB usually served as a booster for plant growth at its initial life stage (Bashan et al. 2004). These PGPB are not known to survive for long periods in dry soil either (Bashan 1999; Bashan et al. 1995, 1999). In addition, the desert soil we used contained enough AM inoculum potential to yield AM colonization of most plants with time, even if not directly inoculated as discussed earlier.

In summary, screen-house reared cardon cacti can be directly transferred to the field if the soil is treated with adjacent young legume nurse trees, mainly mesquite, that also serve as bush cover to reduce soil erosion. Additional treatments such as growth-promoting microorganisms and compost can amplify the effect, mainly with mesquite, or else attenuate it. In our study, the four species of trees survived and grew well under field conditions for 30 months. Cardon–mesquite association supplemented with compost and inoculation with growth-promoting microorganisms is the promising venue for desert reforestation.

Acknowledgments We thank Claudia Rojas, Manuel Moreno, and Luis Leyva for technical assistance in establishing the initial stages of the field studies and Rocio Villalpando, Juan-Pablo Hernandez, Elsa Samarano, and Diana Arizmendi for taking plant measurements and for their help in hand irrigation of the field experiments for 2 years. We thank Jose-Luis Leon de la Luz for botanical advice and Luz de-Bashan for organizing the manuscript and assisting in organization of parts of the field work. This study was mainly supported by Consejo Nacional de Ciencia y Tecnologia of Mexico (CONACYT, contract #50052-Z) and partly funded by The Bashan Foundation, USA. Yoav Bashan participated in this study in memory of the late Avner Bashan of Israel.

References

Arriaga L, Maya Y, Diaz S, Cancino J (1993) Association between cacti and nurse perennials in a heterogeneous tropical dry forest in northwestern Mexico. J Veg Sci 4:349–356. doi:10.2307/3235593

Bacilio M, Hernandez JP, Bashan Y (2006) Restoration of giant cardon cacti in barren desert soil amended with common compost and inoculated with *Azospirillum brasilense*. Biol Fertil Soils 43:112–119. doi:10.1007/s00374-006-0072-y

Banerjee MJ, Gerhart VJ, Glenn EP (2006) Native plant regeneration on abandoned desert farmland: effects of irrigation, soil preparation and amendments on seedling establishment. Restor Ecol 14:339–348. doi:10.1111/j.1526-100X.2006.00142.x

Bashan Y (1999) Interactions of *Azospirillum* spp. in soils: a review. Biol Fertil Soils 29:246–256. doi:10.1007/s003740050549

Bashan Y, Puente ME, Rodriguez-Mendoza MN, Toledo G, Holguin G, Ferrera-Cerrato R, Pedrin S (1995) Survival of *Azospirillum brasilense* in the bulk soil and rhizosphere of 23 soil types. Appl Environ Microbiol 61:1938–1945

Bashan Y, Rojas A, Puente ME (1999) Improved establishment and development of three cacti species inoculated with *Azospirillum brasilense* transplanted into disturbed urban desert soil. Can J Microbiol 45:441–451. doi:10.1139/cjm-45-6-441

Bashan Y, Davis EA, Carrillo-Garcia A, Linderman RG (2000a) Assessment of VA mycorrhizal inoculum potential in relation to the establishment of cactus seedlings under mesquite nurse-trees in the Sonoran Desert. Appl Soil Ecol 14:165–176. doi:10.1016/S0929-1393(00)00050-0

Bashan Y, Gonzalez LE, Toledo G, Leon de La Luz JL, Bethlenfalvay GJ, Troyo E, Rojas A, Holguin G, Puente ME, Lebsky VK, Vazquez P, Castellanos T, Glazier E (2000b) A proposal for conservation of exemplary stands of the giant cardon cactus (*Pachycereus pringlei* [S. Wats Britt. & Ross]) in Baja California Sur, Mexico. Nat Areas J 20:197–200

Bashan Y, Hernandez JP, Leyva LA, Bacilio M (2002a) Alginate microbeads as inoculant carrier for plant growth-promoting bacteria. Biol Fertil Soils 35:359–368. doi:10.1007/s00374-002-0481-5

Bashan Y, Li CY, Lebsky VK, Moreno M, de-Bashan LE (2002b) Primary colonization of volcanic rocks by plants in arid Baja California, Mexico. Plant Biol 4:392–402. doi:10.1055/s-2002-32337

Bashan Y, Holguin G, de-Bashan LE (2004) *Azospirillum*–plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). Can J Microbiol 50:521–577. doi:10.1139/w04-035

Bean TM, Smith SE, Karpiscak MM (2004) Intensive revegetation in Arizona's hot desert. The advantages of container stock. Native Plants J 5:173–180

Brundrett M, Melville L, Peterson L (eds) (1994) Practical methods in mycorrhiza research. Mycologue, Sidney

Carrillo-Garcia A, Leon de la Luz JL, Bashan Y, Bethlenfalvay GJ (1999) Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran Desert. Restor Ecol 7:321–335. doi:10.1046/j.1526-100X.1999.72027.x

Carrillo-Garcia A, Bashan Y, Bethlenfalvay GJ (2000a) Resource-island soils and the survival of the giant cactus, cardon, of Baja California Sur. Plant Soil 218:207–214. doi:10.1023/A:1014953028163

Carrillo-Gracia A, Bashan Y, Diaz-Rivera E, Bethlenfalvay GJ (2000b) Effects of resource - island soils, competition, and inoculation with *Azospirillum* on survival and growth of *Pachycereus pringlei*, the giant cactus of the Sonoran Desert. Restor Ecol 8:65–73. doi:10.1046/j.1526-100x.2000.80009.x

Drezner TD (2006) The regeneration of a protected Sonoran Desert cactus since 1800 A.D. over 50, 000 km^2 of its range. Plant Ecol 183:171–176. doi:10.1007/s11258-005-9015-1

García-Carreño FL (1993) Peroxidase activity in the xerophytic "Cardón" (*Pachycereus pringlei*), a cactaceae of the Sonoran Desert of Mexico. J Plant Physiol 142:274–280

Garner W, Steinberger Y (1989) A proposed mechanism for the formation of 'Fertile Islands' in the desert ecosystem. J Arid Environ 16:257–262

Grover HD, Musick HB (1990) Mexico, U.S.A.: an analysis of desertification processes in the American southwest. Clim Change 17:305–330. doi:10.1007/BF00138373

Halvorson JJ, Bolton H Jr, Smith JL, Rossi RE (1994) Geostatistical analysis of resource islands under *Artemisia tridentata* in the shrub-steppe. Great Basin Nat 54:313–328

Leyva LA, Bashan Y (2008) Activity of two catabolic enzymes of the phosphogluconate pathway in mesquite roots inoculated with *Azospirillum brasilense* Cd. Plant Physiol Biochem 46:898–904. doi:10.1016/j.plaphy.2008.05.011

Marsh BA (1971) Measurement of length in random arrangements of lines. J Appl Ecol 8:265–267. doi:10.2307/2402144

Neilsen GH, Hogue EJ, Neilsen D, Zebarth BJ (1998) Evaluation of organic wastes as soil amendments for cultivation of carrot and chard on irrigated sandy soils. Can J Soil Sci 78:217–225

Nobel PS (1988) Environmental biology of agaves and cacti. Cambridge University Press, Cambridge

Nobel PS (1996) Ecophysiology of roots of desert plants, with special emphasis on agaves and cacti. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots, the hidden half, 2nd edn. Marcel Dekker, New York, pp 823–844

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, Bashan Y, Li CY, Lebsky VK (2004a) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. Plant Biol 6:629–642. doi:10.1055/s-2004-821100

Puente ME, Li C, Bashan Y (2004b) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biol 6:643–650. doi:10.1055/s-2004-821101

Rincón E, Huante P, Ramírez Y (1993) Influence of vesicular–arbuscular mycorrhizae on biomass production by the cactus *Pachycereus pecten-aboriginum*. Mycorrhiza 3:79–81. doi:10.1007/BF00210697

Rose SL (1981) Vesicular–arbuscular endomycorrhizal associations of some desert plants of Baja California. Can J Bot 59:1056–1060. doi:10.1139/b81-144

Roundy BA, Heydari H, Watson C, Smith SE, Munda B, Pater M (2001) Summer establishment of Sonoran Desert species for revegetation of abandoned farmland using line source sprinkler irrigation. Arid Land Res Manag 15:23–39. doi:10.1080/153249801300000798

Scott K (2006) Effect of heat on the dormancy and viability of *Parkinsonia* seeds: implications for management. Ecol Manag Restor 7:153–156. doi:10.1111/j.1442-8903.2006.280_6.x

Stehouwer RC, Macneal K (2003) Use of yard trimmings compost for restoration of saline soil incineration ash. Compost Sci Util 11:51–60

Stutz JC, Copeman R, Martin CA, Morton JB (2000) Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa. Can J Bot 78:237–245. doi:10.1139/cjb-78-2-237

Titus JH, Aniskoff LB, Griffith J, Garrett L, Glatt B (2003) Depth distribution of arbuscular mycorrhizae associated with mesquite. Madroño 50:28–33

Valiente-Banuet A, Ezcurra E (1991) Shade as a cause of the association between the cactus *Neobuxbaumia tetetzo* and the nurse plant *Mimosa luisana* in the Tehuacán Valley, Mexico. J Ecol 79:961–971. doi:10.2307/2261091

Vierheilig H, Coughlan A, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007