

# Growth response of Mediterranean herbaceous swards to inoculation with *Azospirillum brasilense*

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## Abstract

A study was conducted on the effect of the rhizobacterium *Azospirillum brasilense* strain Cd on the production of herbaceous swards growing at 2 rangeland habitats in Israel. One habitat was the semiarid zone (<300 mm annual rainfall, calcareous soil on rocky slopes) while the other was a typical Mediterranean zone (~600 mm annual rainfall, karstic rock covered with terra rossa soil). The inoculum was applied in water suspension at a concentration of  $10^8$  colony forming units (CFU)  $\text{ml}^{-1}$ . The effect of inoculation was compared with P-fertilizer application at a rate of 5 g/m<sup>2</sup>. The same treatments were also applied on potted soil from the 2 sites. The semiarid ecosystem showed a strong response to *Azospirillum* inoculation, to P-fertilizer and to the combination of these 2 treatments, with aerial biomass production increasing by approximately fourfold in the treatments as compared with the control. The response to inoculation or P-fertilization was similar, with no interaction or additive effect noted for the combined treatment. At the Mediterranean site, the response to inoculation or P-fertilization alone was variable, with only a moderate effect on biomass production as compared with the control; however, the inoculation-fertilization interaction was highly significant and doubled biomass production. In the greenhouse experiment, the response to inoculation or fertilization was significant and the biomass production at the end of the growing season was approximately 50% higher than in the control. At both sites, standing biomass was greater in the treated plots already at early stages of growth, thereby potentially lengthening the effective grazing season. It is suggested that inoculation with *Azospirillum brasilense* on a commercial scale may offer a means of increasing rangeland production without resorting to costly and ecologically unfavorable fertilizer application.

**Key Words:** *Azospirillum*, inoculation, rangeland improvement, fertilization

Enrichment of deficient soils with fertilizers is a well-known, if not commonly practiced, range improvement technique. However, its interaction with local conditions can give rise to economic and/or ecological problems (Wight and Black 1979). This has prompted the search for alternative means capable of increasing range production without generating negative consequences.

Nitrogen-fixing bacteria of the genus *Azospirillum* have been suggested as an alternative or supplement to chemical fertilization (Baldani et al. 1983, Hegazi et al. 1983, Kapulnik et al. 1981, Mertens and Hess 1984, Reynders and Vlassak 1982). The plant growth-promoting effects of *A. brasilense* inoculation have been studied mainly with crop plants in field and pot experiments,

usually in combination with different levels of N, P, and K fertilization (Sarig et al. 1988, Sumner 1990). The possible effects of inoculation with *Azospirillum* on plant growth can be explained by its effect on root development. Inoculation has been shown to increase the total root surface under laboratory conditions (Fallik et al. 1988, Sumner 1990), thereby increasing potential mineral and water uptake by the plants (Lin et al. 1983, Sarig et al. 1988). In addition, *Azospirillum* has been shown to increase nitrogen fixation in the rhizosphere, thus enhancing the overall environment conducive to plant growth. In many cases, *Azospirillum* inoculation promoted growth and crop yield of agronomically important forage, grain grasses, and legumes (Okon 1985, Umali-Garcia et al. 1980, Sumner 1990). The bacteria may also affect plant growth by increasing nitrogen uptake in low-nitrogen soils and, more importantly, by producing plant growth substances such as indole-3-acetic acid (IAA) (Barbieri et al. 1986, Fallik et al. 1989). In many field experiments it has been demonstrated that *Azospirillum* inoculants do not cause either environmental hazard (Fages, 1992) or any health problems in the plants (Okon et al. 1987).

The work reported here represents one of the first attempts to evaluate the effect of *Azospirillum* inoculation on the productivity of natural range vegetation. The response of natural pasture growing in 2 different habitats in Israel—semiarid (northern Negev) and Mediterranean climate (Mt. Carmel)—to inoculation with *Azospirillum brasilense* was compared with the effect of phosphatic fertilizer. The potential of using bacteria inoculation as a technique of range improvement is discussed.

## Materials and Methods

Bacterial strain *Azospirillum brasilense* Cd (ATCC 29729), isolated from the roots of *Cynodon dactylon*, was used in all experiments.

### Experimental Sites

The Lehavim site (LV) is located 15 km north of Be'er Sheva (latitude 31° 22'N, longitude 34° 50'E, elevation 400–450 m above sea level) in a semiarid region. The site receives 275 mm average annual precipitation and is covered by Rendzina soil (Rendoll) rich in loess. This soil, like most soils in the Negev, is considered deficient in all major minerals (Noy-Meir and Harpaz 1978).

The Ramat Hanadiv (RH) site is located at the southern tip of Mt. Carmel (latitude 32° 35'N, longitude 35° 00'E, elevation 120 m) in a typically Mediterranean climate. The area receives 550–600 mm average annual rainfall and the rock-soil complex is composed mainly of hard limestone and P-deficient terra rossa (Kaplan 1989).

Preliminary observations showed relatively low available phosphate content at the experimental sites—an average of 6 and 13 mg

The authors wish to thank the Foundation for Ecology of the Jewish National Fund, the Range Management Advisory Board, and Pioneer France-Mais, Aissone, France for providing support for this work. We are grateful to Rafi Yonatan for the field assistance and to No'am Seligman for valuable comments on an earlier version of the manuscript.

Manuscript accepted 4 Jul. 1993.

P/kg soil at Ramat Hanadiv and Lehavim, respectively.

The vegetation of both regions is characterized by shrubs: dominant species in the Mediterranean region are *Sarcopoterium spinosum* (L.) Spach, *Pistacia lentiscus*, and *Calycotome villosa* (Poiret) Link. In the semiarid region, *Sarcopoterium spinosum* (L.) spach and *Coridothymus capitatus* (L.) Reichb are predominant. These shrubs are generally unpalatable to most livestock. The open soil between shrubs is populated by a very diverse community of herbaceous vegetation that develops after the first rainfall (January–February) and withers at the end of spring (end of April–May). It is the latter vegetation that is most important as forage for livestock, and the experiments were conducted in patches of this vegetation. The herbaceous community in Ramat Hanadiv was dominated by the species: *Avena sterilis*, *Brachypodium distachyon*, *Synelcosciadium carmeli*, and *Anagallis arvensis*. In Lehavim the most common species were: *Aegilops* sp., *Rostraria cristata*, *Stipa capensis*, *Anthemis palestina* and *Paronychia argenta*.

### Field Experiments

Both sites were located in hilly areas. The experiments were initiated in the Mediterranean habitat (RH) in 1989 and continued throughout 1990 and 1991. At the semiarid site (LV), experiments ran for 2 years: 1990 and 1991. Each experiment consisted of 4 treatments:

1. **Treatment A:** was composed of inoculation with *A. brasilense*, at a concentration of  $10^8$  colony forming units (CFU)/ml in 2 liters of 34 mM phosphate buffer (pH 6.1) per plot (4 × 4m). The inoculum was applied in water suspension spread uniformly over the plots by a sprayer. Bacteria application followed the first heavy rains (approx. 10 mm rainfall), just before the next storm (usually in December). The amount of water added by the bacteria application was negligible (less than 1 mm) as compared with the annual rainfall (275 mm).

2. **Treatment P:** consisted of fertilization with 85% phosphoric acid (0-61-0), containing 61% P<sub>2</sub>O<sub>5</sub> (Haifa Chemicals, Israel), equivalent to 50 kg P/ha and applied after the first rains. The application technique was similar to that described for Treatment A.

3. **Treatment A+P:** Both inoculation and fertilization (i.e., combination of treatments 1 and 2)

4. **Treatment C:** Control—that received only 2 liters phosphate buffer.

Each treatment was repeated on the same plot in years (3 in RH and 2 in LV).

### Preparation of Plots

The experimental areas were cleared of perennial shrubs and 16-m<sup>2</sup> plots were established. To prevent grazing (by wildlife at Ramat Hanadiv and sheep at Lehavim), the plots were fenced. Herbage yield was measured 4 to 5 times throughout the growing season (February–April) by harvesting all herbaceous vegetation in 5 quadrats (25×25 cm each) distributed randomly within the plot. Plant samples were dried at 80° C in an air-forced oven for 3 days and subsequently weighed.

### Greenhouse Experiments

The same 4 treatments were also applied to plants growing in the greenhouse. Each treatment group consisted of 24 pots with 0.5 kg terra rossa soil from the Ramat Hanadiv site, from which plants and seeds had been removed. In each pot, 0.2 g of a seed mixture collected from the experimental sites was planted. The sown seeds (mainly *Avena sterilis*, *Brachypodium distachyon*, *Synelcosciadium carmeli* and *Anagallis arvensis*.) were inoculated with 5 ml of  $10^8$  (CFU/ml<sup>-1</sup> *Azospirillum*. The amount of P<sub>2</sub>O<sub>5</sub> added was equivalent to 50 kg P/ha. Irrigation was performed as necessary to

maintain pot moisture throughout the entire growing period. Plants from 6 pots of each treatment were harvested and oven-dried on 4 different dates.

### Statistical Analysis

Field results were analyzed using the GLM procedure (Freund and Littell 1980). Significance at the = 0.05 was considered indicative of true differences according to Duncan's Multiple Range Test.

### Results

The effects of inoculation with *Azospirillum* on dry matter biomass yield in the semiarid ecosystem are presented in Figures 1 and 2. During the first growing season (January–April 1990), pasture production in the inoculated plots was statistically higher than that of the control at each sampling date (Fig. 1). At the peak

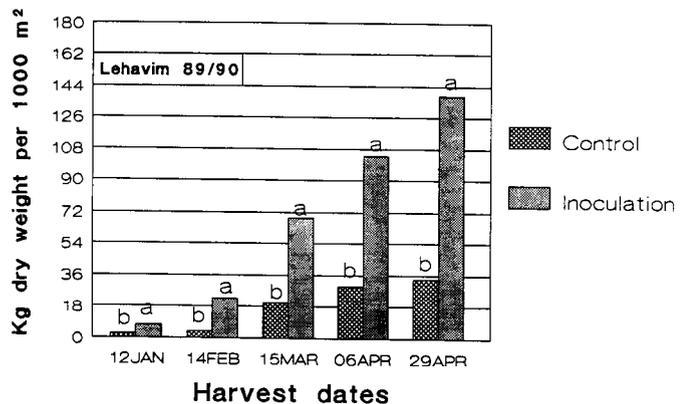


Fig. 1. Effect of inoculation with *Azospirillum brasilense* Cd on the production of natural pasture in the northern Negev (Lehavim)—1989/90. Different letters at each sampling date indicate significant differences at  $p < 0.005$  in Duncan's Multiple Range Test.

of the growth season (end of April), 1,350 kg dry matter/ha were produced in the inoculated plots, as compared with 340 kg dry matter/ha for the controls (i.e., an increase of about 350%). Inoculation apparently influenced pasture productivity mainly during the latter part of the grazing season (mid-February–end of April). The rate of dry matter accumulation in the treated plots was 15 kg/ha/day, as compared with 4.2 kg/ha/d for the control plots.

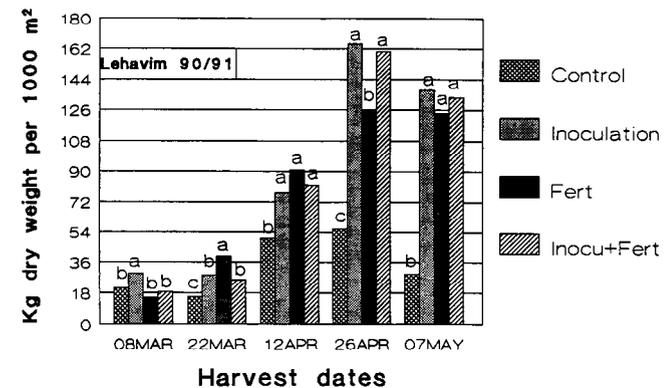


Fig. 2. Effect of inoculation with *Azospirillum brasilense* Cd and phosphate fertilization on the production of natural pasture in the northern Negev (Lehavim)—1990/91.

During the second growing season (1991), phosphate fertilization was added to the experiment. Pasture production was significantly higher in all treatments as compared with the control (Fig. 2). At the peak of biomass production, the yields of the inoculated plots, the fertilized plots, and the plots that had undergone inoculation + fertilization were 1,650, 1,200, and 1,600 kg dry matter/ha, respectively (Fig. 2), or 295%, 214%, and 286% of the control yield (560 dry matter/ha), respectively. Differences between treated and control plots showed up only at the second sampling (end of March). The rate of dry matter accumulation during the main growing season (beginning of March–end of April) was maintained at 29.5, 23.0, 28.3, and 7.3 kg dry matter/ha/d for the combined (A+P) treatment, P-fertilization (P), *Azospirillum* inoculation (A) and the control (C), respectively.

In the Mediterranean shrubby ecosystem (RH), pasture yields from the 4 treatments were not statistically different for the first season (1989). The maximum average yield obtained for this year was 850 kg dry matter/ha and the standard deviations were high.

In the second season (1989/1990), throughout most of the growth period, the yield for all treatments improved significantly as compared with the control (Fig. 3). But at the end of the growing

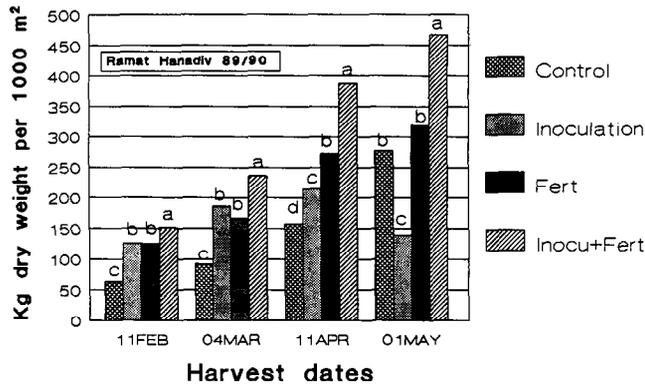


Fig. 3. Effect of inoculation with *Azospirillum brasilense* Cd and phosphate fertilization on the production of natural pasture in Mt. Carmel (Ramat Hanadiv)—1989/90.

season, the only treatment showing a significant increase over the control was that of inoculation + P-fertilization. The decrease in the effect observed with the inoculation treatment in May (Fig. 3) is explained by damage caused by a selective gazelle grazing after the defending fence was left open.

At the peak of the 1990 growing season (April), dry matter yields were 2,150, 2,730, and 3,880, and 1,580 kg/ha in the inoculation, fertilization, inoculation + fertilization, and control treatments, respectively (i.e., an increase of 136%, 173%, and 246% in response to the treatments). The respective rates of dry matter accumulation were 39, 25, 15, and 15 kg/ha/d.

Results of the third year (1991) were similar to those of the second (Fig. 4). In late April, dry matter yield in the control plots was 1,430 kg/ha, inoculation – 1,670 kg/ha, fertilization – 2,250 kg/ha, and inoculation + fertilization 3,590 – kg/ha (117%, 157% and 251% of the control, respectively).

The rates of dry matter accumulation from mid-March to late April 1991 were 52, 32, 24, and 20 kg/ha/d for the combined treatment, fertilization, inoculation, and control plots, respectively.

In the greenhouse experiments (Fig. 5), the first and second harvests of the control treatment showed statistically lower yields than those of the other treatments. By contrast, in the third harvest, the inoculation and combined treatments resulted in significantly higher yields, as compared with the control and fertilization treat-

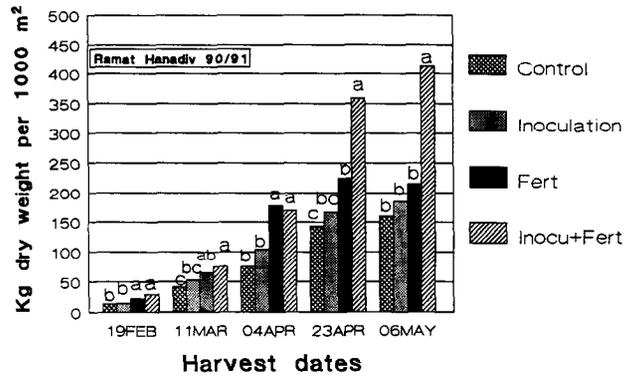


Fig. 4. Effect of inoculation with *Azospirillum brasilense* Cd and phosphate fertilization on the production of natural pasture in Mt. Carmel (Ramat Hanadiv)—1990/91.

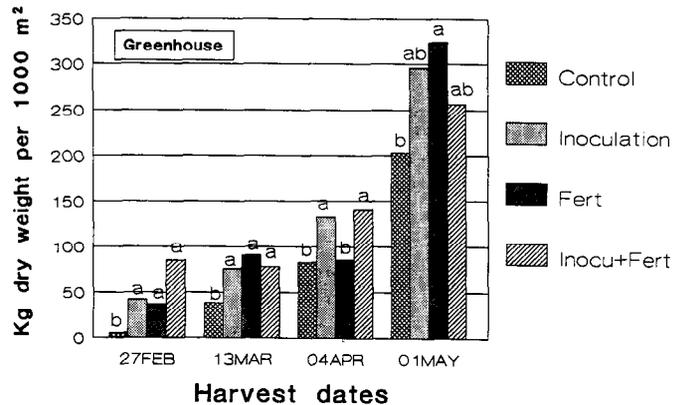


Fig. 5. Effect of inoculation with *Azospirillum brasilense* Cd and phosphate fertilization on the yield of herbaceous vegetation in pots (Greenhouse)

ments. However, just after the end of the growing season (beginning of May), only the fertilization treatment was statistically different from the control, with only slight differences for inoculation alone and fertilization + inoculation treatments.

## Discussion

The findings reported in this paper indicate strongly that inoculation with *Azospirillum brasilense* bacteria has the potential of becoming an applicable range improvement technique under various ecological conditions. The effects of *Azospirillum* inoculation and phosphate fertilization on pasture yields varied from one experimental site to the next. In the semiarid ecosystem, response to inoculation with *Azospirillum* was very strong for both years. The accumulation rate of dry matter was 3.8 times higher than the control. At the peak of the growing season (end of April) the effect of inoculation was significantly higher than that of fertilization, while the yield with the combined treatment did not differ significantly from that of inoculation alone. In other words, inoculating the range with *Azospirillum* is a feasible alternative to expensive fertilization in semiarid habitats.

Bacterial inoculation + phosphate fertilization yielded 100 kg dry matter/ha (minimum biomass for grazing livestock) 4 to 5 weeks earlier than did the untreated plots in the northern Negev. This is economically significant because it allows the farmer to save on supplemental fodder used normally throughout the end of

autumn and the beginning of winter when there is no pasture herbage on the range (Perevolotsky et al. 1991).

Unlike the experimental plots of the semiarid region, those in Ramat Hanadiv had been completely covered with a dense stand of perennial shrubs that were removed during the first season (September 1988). The cleared, newly exposed plots were, most certainly, lacking in seeds of herbaceous species, resulting in a low herbage yield the following season, and, consequently, no treatment effect. During the second and third experimental seasons, the rate of herbaceous pasture yield accumulation was clearly affected by inoculation or fertilization, but the highest significant effects were obtained with the combined treatment (2.5- to 2.6-fold increase in dry matter accumulation rate over the control), when compared with inoculation alone (1.2-fold) or with fertilization alone (1.6-fold).

In the Mediterranean terra rossa soil, the main factor limiting herbage production is phosphate, rather than nitrogen or water (Henkin et al. 1990). It is assumed that the inoculation + phosphate fertilization gave the highest production values due to combined effect of elevated soil phosphate levels and increased root surface area (due to *Azospirillum*), resulting in an increase in overall phosphate uptake by the plants (Okon 1985). Fertilization alone raised, most certainly, the phosphate level in the soil and affected growth, but less than the combination treatment. Similar results were obtained using inoculation alone at low phosphate levels.

Generally speaking, results similar to those observed in the field were obtained in the greenhouse. Treatments increased biomass production significantly as early as the first sampling date, indicating that inoculation promotes the growth rates of individual plants. In the greenhouse, peak biomass accumulation appeared later in the season as compared with the field, due, most certainly, to the abundant water supply in the former during the period of plant growth.

The study of pasture improvement using growth-promoting bacteria as inoculant is still nascent. There is not enough information on the biological effects of *Azospirillum* inoculation or on the adequacy of its application techniques. It is, therefore, too early to provide an economic analysis of this technique or to compare fertilization and inoculation efficiencies. Nevertheless, the use of *Azospirillum* inoculation appears to be much cheaper than fertilization based on a comparison of the cost of its application in agricultural crops such as corn, sorghum, and sunflower as compared with NP fertilization (Okon 1985). Whether the benefits suffice to justify inoculation of rangelands is not yet clear and requires further investigation. Nonetheless, inoculation has been shown to have no hazardous effects on the environment or the plants (Fages 1992), making it an ecologically sound alternative to fertilization.

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