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# REVIEW

# AGRONOMIC APPLICATIONS OF *AZOSPIRILLUM*: AN EVALUATION OF 20 YEARS WORLDWIDE FIELD INOCULATION

YAACOV OKON[1]* and CARLOS A. LABANDERA-GONZALEZ[2]

[1]Department of Plant Pathology and Microbiology, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel and [2]Laboratorio de Microbiologia de Suelos y Control de Inoculantes, Direccion de Suelos y Aguas, Direccion General de Recursos Naturales Renovables, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay

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**Summary**—By evaluating worldwide data accumulated over the past 20 years on field inoculation experiments with *Azospirillum*, it can be concluded that these bacteria are capable of promoting the yield of agriculturally-important crops in different soils and climatic regions. Various strains of *A. brasilense* and *A. lipoferum* have been used to inoculate cultivars of different species of plants. It is however difficult to accurately estimate the percentage of success due to *Azospirillum* inoculation. The data indicates 60–70% occurrence of success with statistically significant increases in yield of the order of 5–30%. Successful inoculation experiments appear to be those in which the researchers have paid special attention to the optimal number of cells of *Azospirillum* in the inoculant, using inoculation methods where the optimal number of cells remained viable and available to colonize the roots. Furthermore, experiments taking into consideration the potentialities and limitations of this technology have been better able to explain successes and failures. The different formulations (analogous to those of rhizobia) of the genus *Azospirillum*, irrespective of their form of application and their mode of action on the plant, are indeed inoculants. The term biofertilizer is not appropriate as it does not replace fertilizer but improves their utilization. We very strongly suggest the implementation by regulatory authorities of quality control on commercial *Azospirillum* inoculants.

## INTRODUCTION

This review is based on the presentations and panel discussions that took place during a workshop entitled "Agronomic Applications of *Azospirillum*", organized by the Laboratory of Soil Microbiology and Inoculant Control held in Montevideo, Uruguay, from 16 to 20 August 1993.

The purpose of the workshop was to review and summarize available results from field experiments and to reach conclusions and recommendations on the feasibility of the agronomic use of this microorganism.

Participants in the workshop were from various research groups from Argentina, Israel and Uruguay with experience in field inoculation with *Azospirillum*. A list of participants and their affiliations is presented in the Appendix at the end of this review.

### *Properties of Azospirillum*

Bacteria of the genus *Azospirillum* are $N_2$-fixing organisms living in close association with plants in the rhizosphere. Five species of *Azospirillum* have been described to date: *A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraeferens* and *A. irakense*. They are Gram-negative, vibrio or spirillum-shaped and 1 μm dia, possessing peritrichous flagella with short wavelengths used for swarming and a polar flagellum used for swimming. Poly-β-hydroxybutyrate granules fill most of the bacterial cell and colonies develop a pink pigment. *Azospirillum* proliferates under both anaerobic and aerobic conditions, but it is preferentially microaerophilic in the presence or absence of combined $N_2$ in the medium. For details on the taxonomy, physiology and genetics of *Azospirillum*, the reader is referred to Dobereiner and Pedrosa (1987), Elmerich *et al.* (1992), Gillis and Reinhold-Hurek (1994), Hartmann and Zimmer (1994), Michiels *et al.* (1994) and Okon (1994).

### *Effects on Plants*

Associative $N_2$ fixation by *Azospirillum* is of less agronomic significance than initially expected. One of the principal mechanisms of growth promotion is related to the capability of *Azospirillum* to produce plant growth promoting substances. In most plant species studied bacterial colonization takes place mainly in the root elongation zone.

*Author for correspondence.

*Azospirillum* stimulates the density and length of root hairs, the rate of appearance of lateral roots and root surface area. The intensity of its effects on root morphology is dependent on the plant species and cultivar and, most importantly, on the concentration of the *Azospirillum* inoculum. In most cases, the optimum concentration is about $1 \times 10^7$ colony forming units (cfu) seed$^{-1}$ or seedling$^{-1}$.

*Azospirillum* inoculation affects the concentrations of free indole-3-acetic acid, and indole-3-butyric acid, as well as the specific respiration rates and specific activities of enzymes involved in the tricarboxylic acid cycle and the glycolysis pathway in roots of maize and other plants. These effects on root morphology and physiology cause roots to take up more water and mineral nutrients resulting in faster plant growth. Under appropriate agronomic conditions, these processes will increase crop yield. The effect of *Azospirillum* inoculation seems to be determined mainly at the early stages of plant development during the first weeks after optimal colonization of roots, see Okon (1985), Dobereiner and Pedrosa (1987), Dell Gallo and Fendrik (1994) and Fallik *et al.* (1994).

## FIELD EXPERIMENTS

### *General Observations*

The accumulated data originating from *Azospirillum* inoculation experiments are summarized below according to countries and research groups. Particular emphasis is given, when pertinent, to descriptions of inoculum preparation and inoculation techniques, soil and climatic conditions, experimental layout and, most importantly, yield data expressed as a percentage increase due to inoculation. For details on plant development, components of yield and yield values, the reader is referred to the published source or the researcher (in the case of unpublished data). For complementary evaluations of the field data, the reader is referred to Okon (1985), Okon *et al.* (1988), Sumner (1990), Wani (1990) and Fages (1994).

More details are given of work presented at the workshop in South America.

### *Summary of Results*

#### *U.S.A.*

Most of the work on *Azospirillum* inoculation was carried out by a group from Gainesville, Florida, under an A.I.D. project (1975–1984) entitled "Nitrogen Fixation by Associative Grass–Bacteria Systems" (Smith *et al.*, 1984). In a first series of experiments (1974–1976) higher dry matter was obtained from grasses inoculated with *A. brasilense* than uninoculated controls, for *Pennisetum americanum* (pearl millet), *Panicum maximum* (guinea grass) and *Digitaria decumbens*. Results indicated that about 40 kg N ha$^{-1}$ y$^{-1}$ were replaced by inoculation (Smith *et al.*, 1978). In subsequent field studies (Smith *et al.*, 1984) three experiments were made: two in Florida and one in New Mexico. Inoculation was accomplished by mixing live or autoclaved cultures of *A. brasilense* strain Cd just before application with 10% (v/w) peat carrier. Approximately $1.5 \times 10^7$ cells, were applied cm$^{-1}$ of row by metering directly behind the planter shoe onto the seed in the furrow with a peristaltic pump mounted on the planter frame. The seed furrow was immediately closed to prevent inoculum desiccation. Significant yield increases (11–24%) were obtained in Florida with *Sorghum bicolor* and with an interspecific hybrid between *Pennisetum americanum* and *P. purpureum*. No significant responses were obtained with pearl millet in Florida or *Sorghum sudanense* in New Mexico. The researchers concluded that responses to inoculation were erratic, although seen in perspective, most of their results were positive. This was the first group to report that yield increases due to *Azospirillum* may be caused by root proliferation rather than by biological $N_2$-fixation (Tien *et al.*, 1979; Smith *et al.*, 1984).

During 1982 and 1983, inoculation experiments were carried out by Biotechnology General, Rehovot, a U.S.–Israeli company trying to develop *Azospirillum* inoculants, in cooperation with the group at the Hebrew University in Rehovot. In 1982, using *Azospirillum* peat-based inoculant (granules or powder applied to the seed furrow) containing about $5 \times 10^6$ cfu g$^{-1}$ moist soil, significant increases (10–20%) in maize yield were obtained in light soils at a site near Athens, Georgia, and at Rutgers University, New Jersey, mainly at intermediate rates of N-fertilization. This observation was made again in Georgia in 1993. However, in both years in Wisconsin, Kansas, Ohio and Iowa, in heavier soils with no N-fertilization, yields were higher due to inoculation, but were not statistically significant. No apparent effect on yield could be observed due to inoculation as N-fertilization was increased (E. Fallik, pers. commun.).

A report (Olubayi *et al.*, 1992) on field experiments conducted in Utah with different genotypes of Kentucky bluegrass revealed positive responses to inoculation with a vermiculite-based carrier containing $10^9$ cfu g$^{-1}$ *A. brasilense* strain Cd. Inoculated plants exhibited better tolerance to drought than non-inoculated controls.

The Company Genesis Turfs and Forages (P.O. Box 10, Huntsville, UT 84317), is marketing an *A. brasilense* inoculant for grasses; "Azo-Green(TM)" is recommended to improve seedling vigor, stand establishment, root systems, drought resistance and overall plant health (Brochure (C) 1993, The Genesis Group).

#### *India*

During the 1980s, many field inoculation experiments were carried out at 11 centers, under the Indian

Coordinated Crop Improvement Project ICAR and ICRISAT.

Results were summarized in 1986 in the Working Group Meeting on Cereal Nitrogen Fixation held by ICRISAT, in Patancheru (Wani, 1986, 1990).

It seems that the inoculant for the ICAR project was supplied by a laboratory in Delhi (Rao, 1986). It consisted of finely-powdered farmyard manure and soil–peat inoculant (1:1 w/w) (it is not mentioned whether this was prepared in a sterilized carrier) initially containing $1.5 \times 10^{11}$ cfu $g^{-1}$ and after 9 months $10^9$ cfu $g^{-1}$. This is the only report in the literature with such a high cell content in the inoculant. The seeds were inoculated by the slurry method using carboxymethylcellulose as the adhesive. No information is given about the number of *Azospirillum* $seed^{-1}$ at the time of sowing.

The results of multilocational trials with pearl millet, finger millet and sorghum were summarized by Rao (1986) and Wani (1990). In finger millet, *A. brasilense* inoculation caused an average (15%) increase in yield over non-inoculated controls. The application of 10–30 kg N-fertilizer $ha^{-1}$ was found to be suitable for maximum benefit of bacterial inoculation in pearl millet. With sorghum, under different agroclimatic conditions, the average increase following inoculation was about 19% (Rao, 1986).

Another series of inoculation experiments with millet and sorghum were carried out at the ICRISAT Center and other locations in India (Wani, 1990). There was a significant increase in yield (20–30%) in 60% of the experiments.

Based on the data spanning about 10 years, it was concluded that statistically significant yield increases were obtained in up to 60% of the field inoculation trials in India with *Azospirillum* (Wani, 1990).

### *Thailand*

Maize inoculation experiments with different $N_2$-fixing rhizosphere bacteria were carried out during the rainy seasons of 1984–1985 in three regions (Vasuvat *et al.*, 1986). Yield increases observed following inoculation were in the range of 15–35% above non-inoculated controls, whereas fertilizing non-inoculated plots with 125 kg N $ha^{-1}$ increased yield by 53%. It was concluded that inoculation could reduce the use of fertilizer by one-third to one-half. No information was given about inoculant preparation, application method or number of cfu $seed^{-1}$.

### *Israel*

Forty experiments (1979–1986) were carried out, with detailed sampling and measurements of field-grown maize, wheat, sorghum, forage grasses and grains, and forage legumes. Plants were inoculated in the furrow with peat-carrier inoculants using *A. brasilense* strain Cd at the rate of $1 \times 10^7$ cfu $seed^{-1}$ or $plant^{-1}$ (Okon *et al.*, 1988). *Azospirillum* inoculation caused a significant (15–20%) increase in yield in all 7 experiments with *Sorghum bicolor* and in experiments with *Panicum miliaceum* and *Setaria italica* (100% success rate). With maize, in 7 out of 12 experiments the increases (20–30% above non-inoculated controls) were statistically significant.

In summer crops, 75% of the experiments showed significant yield increases, whereas in winter crops (wheat) the response to inoculation was significantly higher by 5–12% in only about half of the trials. When comparing inoculated to non-inoculated plots, the largest differences in yield were obtained when the soils were properly but not excessively fertilized.

In more recent experiments, there has been a significant increase in plant biomass following inoculation with *A. brasilense* Cd in Mediterranean and semiarid range–land habitats. At both sites, the standing biomass of herbaceous swards was greater in inoculated plots at early stages of growth, thereby potentially lengthening the effective grazing season (Zaady *et al.*, 1994).

### *Egypt*

There have been many reports of positive effects on yield following inoculation in combination with organic amendments (straw, compost) to soil by various research groups [reviewed by Sumner (1990)]. In a recent report, a strain of *A. brasilense* N040 was selected for its efficiency as a dominant rhizospheric bacteria under gnotobiotic conditions. Inoculation with strain N040 increased rice yield in the field, in fertilized plots, by 15–20%, at two locations in the Nile delta (Omar *et al.*, 1989).

### *Europe*

***Italy.*** Inoculation experiments were made in the regions of Tuscany and Lombardy from 1979 to 1986 in 50–3500 $m^2$ plots (Favilli *et al.*, 1987). Three strains of *Azospirillum* were used: *A. lipoferum* strain 6P isolated from leaves of *Tillandsia* in Costa Rica; *A. brasilense* B-14 from wheat roots; and *A. lipoferum* M-6 from maize root in Italy. Cells from culture broth were washed and concentrated to $1 \times 10^{10}$ cfu $ml^{-1}$ and mixed with a peat–clay carrier (1:1 w/w) or with soil–bovine manure (1:1 w/w) to a final concentration of $1 \times 10^9$ cfu $g^{-1}$ at 40% humidity. Cell number declined in the carriers to $1 \times 10^6$ cfu $g^{-1}$ after 3 and 6 months, respectively. The seeds were treated with arabic gum (10 ml of a 10% solution $kg^{-1}$ seed) and mixed with the inoculant (30 g $kg^{-1}$ seed). In general, inoculation treatments received 50–65% of the N-fertilizer for the particular field, non-inoculated controls received the same amount of fertilizer, and another control was fully fertilized.

Inoculation of rice, wheat, maize and barley (21 experiments) increased the yield of plants that were fertilized with reduced rates of N, to the same or higher yield than the fully fertilized plots. In 18 experiments, *Azospirillum* inoculation increased yields significantly above non-inoculated controls in the range 3–54%. In general, higher increases were obtained with strains isolated from the same host (homologous strains) (Favilli *et al.*, 1987). However, no comparative experiments were made with the different strains on the different hosts under the same experimental conditions.

Other field experiments, with maize (1984–1992), were made in Italy, Germany and Belgium by various laboratories in association with the company Heligenetics (45030 Gaiba, RO, Italy, Via Provinciale 62 a/12). This company now sells a product for maize that contains a mixture of *A. brasilense* strain Cd (ATCC 29729) and *A. lipoferum* Br-17 (ATCC 29709) with vermiculite as the carrier, or a liquid formulation containing $1 \times 10^9$ cfu $g^{-1}$ or $ml^{-1}$. The inoculants have the brand name "Zea-Nit®". Based on experiments carried out with the product, the company reports consistent increases in yield with respect to non-inoculated controls, at intermediate rates of fertilization. *Azospirillum* inoculation replaces 35–40% of the N-fertilizer without reducing the yield as compared to full fertilization (G. Castro-Videla, pers. commun.).

Significant increases (10–40%) above controls in wheat yields were obtained, mainly with intermediate rates of N-fertilizers by inoculation with *Azospirillum* (Reynders and Vlassak, 1982; Mertens and Hess, 1984; Warenbourg *et al.*, 1987).

***France.*** An *Azospirillum* inoculation project was made in France by Pioneer France Mais, Aussonne, Toulouse, over a 6-year period (1987–1992) in 12 locations with 7 maize hybrids (Celia, Dea, Eva, Licea, Sabrina, Sirena and Volga of Pioneer Hi-Bred Int.) (Fages, 1994). The soils were representative of those used for maize crops in France. The plants were inoculated with *A. lipoferum* strain CRT-1, isolated from the rhizosphere of maize. An inoculant consisting of micro-encapsulated bacterial cells dehydrated in a polymer matrix was applied to the furrow at the time of sowing. Inoculum doses were in the range of $2 \times 10^6$ to $1.5 \times 10^8$ cfu $seed^{-1}$.

The experimental layout consisted of a complete randomized block design. Treatments were: inoculated and control combined with 2–4 N-fertilization rates with 4–6 replicates. Fertilizer enriched with $^{15}N$ was used to determine the percentage of N derived from the fertilizer (NddF) and the real use coefficient of N-fertilizer (RUC). The RUC represents the percentage of N-fertilizer which has been translocated into plants (Fages, 1994).

In all 5 trials where response to N-nutrition was followed by the use of $^{15}N$, *Azospirillum* significantly improved NddF and RUC, clearly demonstrating the improvement in N-nutrition by inoculation. There were increases in early growth, vigour, higher emergence rates and early yield components, such as the number of plants per unit area and the number of ranks per ear due to improved N-nutrition. In some cases, depending on the soil climatic conditions of the field, these early advantages were converted into higher yield. Yield increases were obtained in only 5 experiments with suboptimal rates of N-fertlilization. Earlier flowering dates were recorded in 2 experiments and better drought resistance, as measured by porometry, was observed in 3 experiments.

It was concluded that a positive response to *A. lipoferum* CRT-1 inoculation could be obtained regardless of maize cultivar or soil type. The main effect of inoculation was better use of the N-fertilizer (Fages, 1994). The results on N-nutrition were very consistent and the product was registered in France under the brand name "Azogreen®".

An inoculation experiment with "Azogreen®" in the Agbasar station in Northern Togo, Africa increased maize yield by over 100% under two rates of NPK fertilizer (J. Fages, pers. commun.).

*Latin America*

***Brazil.*** This group carried out 3 field experiments with wheat (cv. Anahuac) from 1983 to 1985 in Sertanejo, Parana, in a wheat region (Baldani *et al.*, 1987). The soil was "terra rossa", pH 5.6, 8.8 $\mu g\ g^{-1}$ P and 0.31% N. The inoculum was prepared by growing *A. brasilense* strains for 24 h in NFb medium with $NH_4Cl$. Granulated peat (6 kg) was mixed with 1 l. of culture and 10 g were added for each metre of furrow. The non-inoculated control was treated with dead cells. No information was given about the number of viable cells in the peat granules at the time of inoculation.

In 1983, no *Azospirillum* strain or N-fertilization rate had any significant effect on grain yield. However, at the grain-filling stage, *A. brasilense* strains Sp-245 and Sp-107st increased the dry weight and N-content of plants significantly above controls. Strain Sp 7-Cd reduced the dry weight and N-content of plants as compared to controls. Similar results were obtained in 1984 at the flowering stage. No data were presented on grain yield. In 1985, only strains Sp-245 and Sp-245 NR- were tested and both significantly increased the total N-content of plants and grain yield above that of the controls.

Two experiments were carried out in Seropedica, Rio de Janeiro (Boddey *et al.*, 1986), one in soil cylinders (60 cm dia and 50 cm depth) with wheat cultivar BA 1146. Soil was sterilized with methylbromide 2 weeks before sowing. The *Azospirillum* population was reduced by this treatment from $10^5$ to $10^2\ g^{-1}\ soil^{-1}$. No information is given as to when the *Azospirillum* population was counted. Each cylinder was inoculated with a 48-h NFb + $NH_4Cl$ culture. No details are given about the number of viable cells from each strain at time of inoculation. In this experiment, both *A. brasilense* strains Sp-245 and Sp 7-Cd increased the grain yield and N-content of plants. Measurements using the $^{15}N$-dilution technique showed that this increase was not due to biological N-fixation.

In a second, parallel experiment with cultivar Cocoraque, carried out in the field in similar soil, strains Sp-245, Sp-246 and *A. amazonense* increased the dry weight and N-content of plants, whereas the effect of strain Sp 7-Cd was lower.

From these two series of wheat field experiments it was concluded that strains (e.g. Sp-245) that were isolated from surface-sterilized roots of wheat ("homologous strain") were more efficient at promoting growth in soils with a high population of *Azospirillum*, whereas strains which were isolated from the surface of roots from other species (Sp 7-Cd, *Digitaria decumbens*) were less effective (Boddey and Dobereiner, 1988).

It is possible based on the consistency of the observations, that strain Sp-245 is more efficient for wheat. However, taking into consideration the fact that there is an optimal inoculum concentration for promoting yield, the number of viable cells of each strain at inoculation time needs to be known.

Nevertheless, these experiments clearly demonstrated the potential for increasing wheat yield, even in soils rich in *Azospirillum* populations.

***Mexico.*** There has been a large field-inoculation effort with wheat and maize by a research group at the University of Puebla (Paredes-Cardona *et al.*, 1988; Caballero-Mellado *et al.*, 1992). In experiments carried out in 1986 and 1987 in a temperate area, wheat was inoculated with various strains of *Azospirillum*. The populations in the peat carrier were $3–5 \times 10^8$ cfu $g^{-1}$. Minutes before sowing, the seeds were inoculated (15 g kg $seed^{-1}$) using arabic gum as adhesive (40 ml kg $seed^{-1}$). Inoculation caused significant increases in yield, from 23 to 63% in 1986 and from 24 to 43% in 1987 (yields in the range 1.5–3.0 t $ha^{-1}$). Best results were obtained with a local *A. brasilense* strain isolated from the rhizosphere of *Brachiaria mutica* (UAP-55) as well as with strain Cd.

On maize, the group reports consistent (1985–1993) increases in grain yield in the order of 30–70%, with savings of 50% on the N-fertilizer, in the states of Puebla, Tlaxcala and Veracruz. Grain yield levels in these areas were 1.5–5.0 t $ha^{-1}$.

In Puebla and Veracruz, the economic effects of the inoculant are being evaluated. Due to favourable responses, in 1992 2000 ha were inoculated, and in 1993 the demand had risen to 5000 ha (M. A. Mascarua-Esparza, pers. commun.). The inoculant is produced as "Biofertilizante para Maiz" by the Centro de Investigaciones en Ciencias Microbiologicas, Microbiologia del Suelo, Universidad Autonoma de Puebla, Apdo Postal 1622, Puebla, Puebla, Mexico.

***Uruguay.*** A project was initiated in 1990 by the Laboratory of Soil Microbiology and Inoculant Control, aimed at determining the potential of increasing crop yields of grain and forage grasses and legumes by inoculation with *Azospirillum*. If a positive effect is observed, the project will establish the optimal composition of inoculant, methods of application and the agronomic conditions that favour the expression of the response in each crop.

In a preliminary field experiment (1992–1993), inoculation with a peat-based *A. brasilense* Cd inoculant applied in the furrow or on the seeds ($1 \times 10^7$ cfu $seed^{-1}$) resulted in the promotion of sorghum yield by 10–15%. Results with maize have been variable.

***Argentina.*** Extensive field inoculation experiments have been carried out by various research groups in Argentina. Most of the results reviewed below were presented at the workshop in Montevideo. This material has been presented mainly at scientific meetings and not in reviewed publications.

*Castelar—Province of Buenos Aires. A. brasilense* increased the yield of wheat (*Triticum aestivum* cv. Marcos Juarez INTA) 33% over non-inoculated controls (Barrios *et al.*, 1986).

Other field experiments were carried out for 3 years in the semiarid pampean region, featuring recurrent problems of water deficit and soil depletion. This region yields an average of 1400 kg wheat grain $ha^{-1}$, as compared to the 2000 kg $ha^{-1}$ obtained in the humid pampas (Rodriguez-Caceres *et al.*, 1994). The wheat cultivars studied were Cochico INTA, Prointa Pigüe and Buck Poncho. The strains used were: *A. brasilense* Az-39 isolated from washed wheat roots (Marcos Juarez, Cordoba); *A. brasilense* Cd; *A. brasilense* Sp-245 (Baldani *et al.*, 1987); and *Bacillus polymyxa* BP NRRC 4317. Seeds were inoculated shortly before sowing with a peat–charcoal inoculant, with 60 ml of 5% "Cellofas A" adhesive being added to 1 kg of seeds. Before sowing, seeds had an average of $4 \times 10^6$ cfu $seed^{-1}$.

The most consistent *Azospirillum* strain, significantly promoting wheat yield over 3 seasons in unfertilized soil, was strain Az-39 and, to a lesser extent, strain Cd. The increases above controls were in the order of 13–30% in all tested cultivars. No significant effects were obtained with strain Sp-245 (tested for 2 years). In soils fertilized with 40 kg N $ha^{-1}$ the bacteria did not promote yield above controls. In general, *Bacillus polymyxa* gave positive results, but less marked than *A. brasilense* Az-39 and Cd (Rodriguez-Caceres *et al.*, 1994). Results with maize over the years have been inconsistent (E. A. Rodriguez-Caceres, pers. commun.).

Nevertheless, Barrios *et al.* (1984) have reported increases of 37% in the yield of maize cv. PixF465 in Pergamino, Province of Buenos Aires, with peat inoculant ($10^8$–$10^9$ cfu $g^{-1}$), containing *A. brasilense* strain Az-5 INTA, isolated from the rhizosphere of maize, Pergamino. With the other two strains, *A. brasilense* Az-3 INTA isolated from maize roots, Castelar, and *A. lipoferum* ATCC 29709, yield increases were not significant.

The effect of *Azospirillum* on the yield of *Setaria italica* cv. Yaguane INTA, was investigated by Di Ciocco and Rodriguez-Caceres (1994), using inoculation methodology as described for wheat (Rodriguez-Caceres *et al.*, 1994). Before sowing, there was an average $10^5$ cfu $g^{-1}$ seed. Strains *A. brasilense* Az-39 and Cd, and *A. lipoferum* Az-30, isolated from the rhizosphere of *Eragrostis* sp. in the Province of Cordoba were tested. In the first season, cv. Yaguane was inoculated with a mixture of the 3 strains. The yield was significantly increased by 30%. In the second season, strains Az-39 and Cd, inoculated individually, increased yields significantly (by about 21%).

*Viedma—Province of Rio Negro*. Inoculation experiments were carried out over 5 seasons (1988–1992). The soil and climatic conditions were semiarid to arid. Annual rainfall varied from 257 to 631 mm, soil pH was 6.5–7.5, with 0.8–2.0% organic matter and 16–28 5 g P $g^{-1}$. Seeds were inoculated with a peat inoculant of *A. brasilense* strain Sp-111, an isolate from the rhizosphere of grasses from Patagonian soils. Significant increases of 1.3- to 2-fold in yield above controls were obtained in wheat cv. Buck Manantial in each of the 5 growing seasons. Yields varied widely according to climatic conditions, which varied enormously from year to year (G. Pozzo-Ardizzi, pers. commun.). No information was given on rate of inoculation and number of *Azospirillum* per seed at sowing time.

*Tucuman—Province of Tucuman*. This group reports very consistent, significant yield increases above non-inoculated, unfertilized controls (C.H. Bellone, pers. commun.). The inoculum was prepared from local undescribed isolates of *Azospirillum*, at least 20% of whose population colonized the "interior" (bacterial counts after surface sterilization of roots) of inoculated roots. Fifteen-day cultures were utilized in liquid form or in peat carriers, apparently because cultures in the stationary phase accumulate more plant growth substances (C. H. Bellone, pers. commun.).

The data on promotion of yield presented by this group are consistent, and the extent of the effect is high. In 6 cultivars of wheat, over 7 seasons (1985–1991), increases (above non-inoculated unfertilized controls) in the order of 15–30% were obtained, equivalent to fertilization with 10 kg N $ha^{-1}$. The response to inoculation increased to 50–60% in inoculated and fertilized (10 kg N $ha^{-1}$) plots.

Similar results were obtained for various cultivars of maize over 6 seasons (1986–1991). Increases due to inoculation were in the range 15–25% in inoculated unfertilized plots, and 40% in inoculated and fertilized (10 kg N $ha^{-1}$) plots (C. H. Bellone, pers. commun.).

*Rio Cuarto—Province of Cordoba*. Inoculation experiments (1987–1990) were carried out with a mixture of *A. lipoferum* strain Wt (ATCC 29708) and *A. brasilense* strain Cd (ATCC 29710). Treatments were: these 2 strains alone (I) or with 10 kg N as urea $ha^{-1}$ (I+N10); fertilized controls (30 and 60 kg N $ha^{-1}$; N30 and N60, respectively); and non-inoculated unfertilized controls. The experimental field was located at the University of Rio Cuarto, in a Typic Hapludox with a sandy clay texture, pH 6.5, 1.6% organic matter and 16 5g P $g^{-1}$ with a field capacity of 16% (M. Fulchieri, unpubl. Ph.D. thesis). Evaluations made at harvest on the same plots over a 3-year period showed similar trends. In Treatment I, the yield, expressed in kg $ha^{-1}$, and the analysis of yield components revealed an increase in the order of 10% (most of it not significant). In Treatment I+N10, yields were 16–31% over the controls (mostly significant) and comparable to Treatment N60. In Treatment N30, the effects on yield were not significant over the control.

*Daireaux—Province of Buenos Aires (Agrobiotec S.A.)*. A field inoculation experiment with 6 maize hybrids was carried out with a commercial inoculant (Zea-Nit, Heligenetics) in the 1992–1993 season, with an annual average rainfall of 800 mm on Hapludox soil, pH 6.4–6.7 with 4.4–6.4 5 g P $g^{-1}$ and 1.38% organic matter.

The seeds were inoculated prior to sowing with a liquid formulation in sterilized substrate containing $5 \times 10^8$ *A. brasilense* Cd and *A. lipoferum* Br-17 (Zea-Nit, Heligenetics), each seed received $5 \times 10^6$ to $1 \times 10^7$ cfu.

Responses were as follows: hybrid AX 788 (Asgrow/Nidera), significant increase of 8.0%; 4F91 (Dekalb), no response; P 3379 (Pionner), 3.4% increase; P 3456 (Pionner), significant increase of 10.5%; 4F37 (Dekalb); significant increase of 10.5%; and 4R160 (Cargill), 5.1% increase (G. Castro-Videla, Agrobiotec S.A., pers. commun.).

*Junin and Open Door—Province of Buenos Aires*. Field experiments were carried out in 1987, 1988 and 1990. The seeds were inoculated by the slurry method at sowing with a peat inoculant, about $1 \times 10^6$ cfu $seed^{-1}$, or over the seeds in the furrow. The *A. brasilense* strains utilized were Az-12 and Az-31 isolated from the rhizosphere of wheat, Az-39 obtained from INTA Castelar, and strain SP-245. In Junin (1989) there were no significant yield increases (8–13%) above controls in grain; in Open Door (1988) no significant increases in yield were obtained by inoculation with any of the strains (M. A. Monzon and I. Garcia, pers. commun.).

## CONCLUSIONS

### *Overall Effects*

By evaluating worldwide data accumulated over the past 20 years on field inoculation experiments with *Azospirillum*, it can be concluded that this bacterium is capable of promoting the yield of agriculturally-important crops in different soils and climatic regions, using various strains of *A. brasilense* and *A. lipoferum* and cultivars of different species of plants. However, it is difficult to estimate accurately the percentage of success due to *Azospirillum* inoculation. The picture emerging from the extensive data reviewed above is of 60–70% successes with statistically significant increases in yield in the order of 5–30%. This estimate is in agreement with Okon *et al.* (1988) and Wani (1990) for experiments in Israel and India, respectively. Sumner (1990) has presented 32 references relating to positive effects in the field and 7 citations (mostly on wheat) of failures to obtain yield increases. He concluded that the responses have been quite substantial, well in excess of the likely costs of inoculation, making this technique highly attractive to the farmer. Furthermore, Fages (1994) proposed that a well-focused strategy of field experimentation could demonstrate an acceptable consistency of agronomic results: for example, the results with sorghum in Israel (Sarig *et al.*, 1984, 1988) and the results with maize in France (Fages, 1994).

References can be found in the literature (Bashan and Levanony, 1990; Sumner, 1990), to the effect that negative or zero responses to inoculation have been arbitrarily suppressed from publication. In reviewing the literature, we have found that most of the research groups involved in field studies with *Azospirillum* have, in effect, reported those "negative" results (Smith *et al.*, 1984; Rao, 1986; Wani, 1986; Baldani *et al.*, 1987; Favilli *et al.*, 1987; Okon *et al.*, 1988; Rodriguez-Caceres *et al.*, 1994, Fages, 1994). We therefore consider the value of 60–70% success valid.

### *Criteria for Evaluation and Interpretation of Field Results*

Early experimentation (1974–1982) (Smith *et al.*, 1984; Wani, 1986) was designed to demonstrate promotion of growth by biological N-fixation (BNF) (Dobereiner and Pedrosa, 1987). Following the observation that plant growth promotion caused by *Azospirillum* inoculation was due to promotion of root growth rather than BNF (Smith *et al.*, 1984; Okon, 1985), experimental objectives and designs changed somewhat. With the new approaches there were relatively more successes, especially under intermediate rates of NPK fertilization. In experiments that evaluated, during plant ontogeny, the percentage of emergence, early components of yield, NPK nutrition (content in plants) and the water status of plants, the percentage of success due to inoculation increased to 80 or 90%. Growth promotion however did not always translate to higher crop yield (Okon *et al.*, 1988; Sumner, 1990; Fages, 1994).

Boddey and Dobereiner (1988), in agreement with Wani (1990), Favilli *et al.* (1987) and Sumner (1990), proposed that strains of *Azospirillum* isolated from "inside" the roots (after surface sterilization) of a host are more efficient in promoting yield when used as inoculum for the homologous host. Whereas from an ecological point of view this hypothesis seems logical, from the data we have reviewed it is difficult to assess whether it is valid and consistent. In general, field experiments have not been designed to answer this questions, i.e. have not involved a comparison of strains under the same field conditions, during various seasons (Sumner, 1990), taking special care to use the same number of *Azospirillum* cells in the inoculant. An optimal inoculum concentration for promotion of root growth has, however, been demonstrated (Okon, 1985; Bashan and Levanony, 1990).

For example, the experiments of Boddey *et al.* (1986) and Baldani *et al.* (1987) showing that the wheat "homologous" *A. brasilense* strain Sp-245 was more efficient for wheat, could not be confirmed by Rodriguez-Caceres *et al.* (1994) in the semiarid pampas of Argentina. By using an equal number of cells in the inoculants, they observed consistent effects on wheat over 3 seasons in various wheat cultivars with the "homologous" strain Az-39. Nevertheless, strain Sp-Cd gave comparable results, while strain Sp-245 was much less efficient. In Argentina, "heterologous" strain Az-39 and Sp-Cd promoted yield of *S. italica*. Many more such discrepancies can be found in the literature.

Successful inoculation experiments appear to be those in which the researchers have paid special attention to the optimal number of cells of *Azospirillum* in the inoculant, using appropriate inoculation methods where the optimal number of cells remained viable and available to colonize the roots. Furthermore, those taking into consideration the potentials and limitations of this technology have been better able to explain successes and failures (Fages, 1994).

### *Field Experiments*

While reviewing the literature on field inoculation experiments with *Azospirillum*, we noticed that there is lack of important information that could explain the success or failure in promotion of yield. The following recommendations are suggested for field experiments with *Azospirillum*: Before a field experiment is conducted, we recommend collecting as much information as possible on the crops history, micro climate, soil conditions of the field, residue management, the yield levels obtained. The chemical treatments to the soil in previous seasons and those prior to sowing are also crucial, as well as those applied to the seed or foliage. Other factors worth monitoring are soil test values including texture and salinity, analyses of nitrates at sowing, nitrification rates, humidity of soil during the growth cycle, soil temperature at sowing and during plant growth, precipitation and temperature extremes, lastly, light intensities should be determined at sowing, during the first month of growth, and at flowering and grain filling.

### *Experimental Details*

Trials should be performed in a representative field using local agrotechniques, to obtain results which will be valid for future utilization by farmers of the region.

The characteristics of the cultivar used, viz. name, source and length of growth cycle, need to be mentioned as well as the drought and diseases resistance.

The inoculant formulation, inoculation technique and specific strain of *Azospirillum* used (number in a local collection) are crucial. The original collection from which it comes (such as ATCC etc.) are often not mentioned by researchers. The researcher must identify the *Azospirillum* positively, by characterizing it via modern taxonomic methods such as cytosine–guanine DNA content, DNA–DNA hybridization and rRNA–DNA hybridization (Gillis and Reinhold-Hurek, 1994). The cultivation method and medium used for *Azospirillum* need to be presented and, most importantly, the number of cells in the inoculant and on the seed surface at the time of sowing. Non-inoculated plants should be treated similarly to inoculated ones, using sterile peat with dead cells on the seeds.

*Statistical Design, Characteristics and Variables to Measure*

The expected effects should be reflected in the level of significance. The trial should be planned in collaboration with a statistician. It is also convenient to have the participation of multidisciplinary experts for the design and execution of the experiment, i.e. microbiologists, crop physiologists, soil scientists etc.

It is recommended to measure the yield (commercial and industrial quality and yield components), NPK content in plant parts at harvest, recovery of $^{15}$N-fertilizer (Fages, 1994), the water status of the plant, measured by steady-state porometry, leaf water potential and canopy temperature (Sarig *et al.*, 1988), and to measure the stages of plant development: (percentage germination); percentage emergence; growth vigour; root systems in seedlings; tillering; flowering time; and appearance of diseases and stresses.

It should be noted that microbiological tests using conventional methodology regarding colonization of the introduced bacteria in the rhizosphere and inside the roots in the field, are very laborious and many times not very informative.

*Comparisons with Experimental Design for Rhizobium Field Inoculation*

Many of the researchers involved in *Azospirillum*–plant associations have been trained as rhizobiologists. It is therefore pertinent in this review to point out similarities and differences between the two systems.

Characteristics of the *Azospirillum*–plant system include agronomic responses of a potentially lower magnitude as compared to those expected in *Rhizobium* inoculation. Consequently, the plots in the experiment should be larger with more replications and the experimental area should be larger with more possibilities of variability, closer monitoring and a larger number of observations and detailed sampling.

The expression of the potential response in both *Azospirillum* and *Rhizobium* systems requires colonization of roots by the introduced bacteria. With *Rhizobium*, there are three possibilities:

(1) No rhizobia are present in the soil belonging to the same inoculation group, resulting in a lack of competition.

(2) With rhizobia capable of nodulating the host effectively, the response to inoculation varies and depends on the characteristics of the native population and the competitive relationships. In this case, local strains should be isolated and used for comparison with the introduced ones.

(3) Rhizobia in the soil, which is only capable of nodulating the host ineffectively and therefore parasitically; the success of establishing an effective symbiosis depends on the saprophytic capability of the introduced strain (Alexander, 1984).

With *Azospirillum*, the interactions of the introduced strain with other rhizosphere microorganisms are still controversial and difficult to evaluate, especially under field conditions. To answer this question, more sophisticated methodology (molecular probes) is needed to study the ecology of *Azospirillum* in the soil and rhizosphere.

The qualitative and quantitative components of selected strains with *Rhizobium* and *Azospirillum* and the potential plant responses are of importance. In *Rhizobium*, the expression of a strain in terms of increased yield depends on the combined effect of two components, each carrying relative weight: the legume host and the given agronomic conditions.

Example 1. In soybean, in soil without a specific native population, a response potential of 800–1000 kg grain ha$^{-1}$ is expected. However, under water stress, the expression of this potential depends on the number of rhizobia in the inoculant and on the inoculation method.

Example 2. In soybean, in the presence of a native effective rhizobial population, the response potential depends on the qualitative and quantitative properties of the introduced strain.

Example 3. In subterranean clover, in the presence of ineffective and parasitic rhizobia, the response potential depends on both the qualitative characteristics of the introduced strain (competitiveness and effectiveness) and the number of rhizobial cells introduced together with the seed.

With *Azospirillum* the importance of the strain appears to be controversial. The concept of specificity or affinity between strain and host has not been proven and there is no agreement in the literature.

An investigation into the potential response of *Rhizobium* in the field requires at least three treatments: (a) non-inoculated control without N; (b) non-inoculated control without N-limitation; and (c) inoculated treatment without N.

For *Azospirillum* there is a need for: (a) a non-inoculated control with intermediate fertilization (NPK) and or water; (b) an inoculation treatment with intermediate fertilization and or water; and (c) a non-inoculated control with unlimited fertilization and water.

In the case of *Rhizobium* one must take all precautions to prevent cross-contamination, whereas *Azospirillum* allows for less strict handling because of the importance of the quantitative component in the expressing of responses (about $10^7$ cfu plant$^{-1}$).

In addition to those variables generally measured with *Rhizobium* (Halliday, 1984), for *Azospirillum* one needs to measure the root surface area, the water status of the plants and the yield component, and to use isotopic techniques to follow the efficiency of fertilizer use.

### *Inoculants and their application.*

The different formulations (analogous to those of *Rhizobium*) of the genus *Azospirillum*, irrespective of their form of application and their mode of action on the plant, are indeed inoculants. The term biofertilizer is not appropriate. *Culture Media.* Based on the reviewed literature, it is apparent that there are no limitations to obtaining cultures with high *Azospirillum* counts: $5 \times 10^9$ to $2 \times 10^{10}$ cfu ml$^{-1}$. The most common medium used is NFb with $NH_4Cl$ or a modification (Dobereiner and Pedrosa, 1987). The addition of growth-promoting substances (yeast extract) and modifications to the N-concentrations in NFb could improve growth. Some researchers emphasize the importance of the cell number but not their physiological state, when used as inoculum. Most laboratories utilize logarithmic or late-logarithmic phase cultures, with fermentation periods of 30–72 h. Other laboratories prefer 10- to 15-day-old cultures in the stationary phase. The objective is to obtain cell structures (cysts) having survival advantages, i.e. cell storage materials and accumulated growth-promoting substances (Sadasivan and Neyra, 1985; C. H. Bellone, pers. commun.; G. Pozzo Ardizzi, pers. commun.).

Reports of positive responses to inoculation have been published without reference to the fermentation methodology used, and it is therefore not yet possible to assess whether one particular fermentation method is more beneficial than another. More detailed studies are required to evaluate the effect of the physiological status of the cell in the formulation of inoculants with respect to: survival in the carrier; survival on the seed, colonization of the rhizosphere; and the amount of plant response. The role that secondary metabolites accumulating in the medium may play on plant growth promotion has been investigated under controlled conditions. The observation has frequently been made that only cells (washed from the medium), and not the cell filtrates, are responsible for promotion of root growth (Okon and Kapulnik, 1986).

*Carriers and formulations.* Problems of *Azospirillum* survival in the inoculants have been examined in carriers such as peat, vermiculite, alginates and liquid formulations (Fages, 1992). A frequent observation is that in peat-based inoculants with $10^9$ cfu g$^{-1}$, the number of viable cells decreases to $10^7$ after 90 days of storage (Favilli *et al.*, 1987). Better results have been obtained for cells in dried microgranulated alginate formulations (Fages, 1992). Survival may be improved by paying attention to the physiological stage of the cells, e.g. high content of PHB, cysts and flocculates (Sadasivan and Neyra, 1985).

Obtaining concentrations of over $1 \times 10^5$ to $1 \times 10^6$ cfu g$^{-1}$ seed is difficult, especially in smaller seeds such as wheat, when using slurries of peat or liquid inoculants on the seeds. The situation is better with larger seeds (maize and sorghum). There may also be differences in cell-harbouring capacity due to the texture of the seed surface (rough, smooth etc.). Nevertheless, positive effects have been reported with the slurry method for wheat, barley and other small-seeded species.

We recommend working with sterilized carriers. Those normally used for *Azospirillum* have been peat, with additives such as charcoal and clays, vermiculite or alginates. Promising results have also been obtained with liquid formulations (G. Castro-Videla, pers. commun.). The use of a culture medium with washed cell suspensions is adequate for experiments under controlled conditions. We recommend determining qualitative and quantitative advantages of the different carriers for survival of the bacteria in the carrier and on the seed.

*Methods of inoculum application.* This is a particularly relevant aspect because of the intended use of *Azospirillum*, i.e. mainly for extensive crops, whereby the use and application of the inoculant must be simplified for the farmer.

The existing application alternatives are: on the seed or in the furrow; in a liquid or solid formulation. With all four possibilities there have been reports of positive results. However, the volumes of inoculant needed to achieve optimal inoculum cell concentrations vary a great deal.

To optimize the application technology, comparative experiments need to be carried out in the field with the different methods of application. With respect to this, there is very little published information.

The characteristics that need to be determined in these comparative experiments are: yield increase; survival of bacteria on the seed; number of cells in the roots of seedlings; and detailed effects of seed treatments (fungicides, herbicides and adhesives) on *Azospirillum* survival.

### *General recommendations for inoculants*

We very strongly suggest the implementation by regulatory authorities of quality control on commercial *Azospirillum* inoculants. Good examples of successful quality control have been reported for *Rhizobium* in Australia and Uruguay (Date, 1965). It is necessary to maintain high standards for *Azospirillum* inoculants with proven efficient strains and cell numbers of the order of $1 \times 10^9$ to $1 \times 10^{10}$ cfu $g^{-1}$ or $ml^{-1}$ and recommendations need to be provided for their proper use by farmers.

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## REFERENCES

Alexander M. (Ed.) (1984) Ecology of *Rhizobium*. In *Biological Nitrogen Fixation, Ecology, Technology and Physiology*, pp. 39–50. Plenum Press, New York.

Baldani V. L. D., Baldani J. I. and Dobereiner J. (1987) Inoculation of field-grown wheat (*Triticum aestivum*) with *Azospirillum* spp in Brazil. *Biology and Fertility of Soils* **4**, 37–40.

Barrios S., Rodriguez-Caceres E. and Damilano A. (1984) Respuesta del maiz a la inoculacion con *Azospirillum* spp. In *Proceedings of III Congreso Nacional de Maiz*, Pergamino, Buenos Aires, Argentina, 6–9 November.

Barrios S., Potenza A. and Lopez M. V. (1986) Utilizacion del *Azospirillum* (diazotrofo rizosferico) en el triticultivo. In *Proceedings of Primer Congreso Nacional de Asociacion de Ingenieros Agronomos de la Zona Norte de la Provincia de Buenos Aires*, Pergamino, Argentina, 6–10 October.

Bashan Y. and Levanony H. (1990) Current status of *Azospirillum* as a challenge for agriculture. *Canadian Journal of Microbiology* **36**, 591–608.

Boddey R. M. and Dobereiner J. (1988) Nitrogen fixation associated with grasses and cereals: recent results and perspectives for future research. *Plant and Soil* **108**, 53–65.

Boddey R. M., Baldani V. L. D., Baldani J. I. and Dobereiner J. (1986) Effect of inoculation of *Azospirillum* spp on nitrogen accumulation by field-grown wheat. *Plant and Soil* **95**, 109–121.

Caballero-Mellado J., Carcano-Montiel M. and Mascarua-Esparza M. A. (1992) Field inoculation of wheat (*Triticum aestivum*) with *Azospirillum brasilense* under temperate climate. *Symbiosis* **13**, 243–253.

Date R. A. (1965) Legume inoculation and legume inoculant production. FAO Report 2012, Rome.

Dell-Gallo M. and Fendrik I. (1994) The rhizosphere and *Azospirillum*. In *Azospirillum–Plant Associations* (Y. Okon, Ed.), pp. 57–76. CRC Press, Boca Raton, Fla.

Di Ciocco C. A. and Rodriguez-Caceres E. (1994) Response of *Setaria italica* to inoculation with *Azospirillum* spp. *Journal of Experimental Agriculture*. In press.

Dobereiner J. and Pedrosa F. P. (1987) *Nitrogen-fixing Bacteria in Non-leguminous Crop Plants*. Springer-Verlag, Berlin.

Elmerich C., Zimmer W. and Vielle C. (1992) Associative nitrogen-fixing bacteria. In *Biological Nitrogen Fixation* (G. Stacey, R. H. Burris and H. J. Evans, Eds). Chapman & Hall, New York.

Fages J. (1992) An industrial view of *Azospirillum* inoculants: formulation and application technology. *Symbiosis* **13**, 15–26.

Fages J. (1994) *Azospirillum* inoculants and field experiments. In *Azospirillum–Plant Associations* (Y. Okon, Ed.), pp. 87–110. CRC Press, Boca Raton, Fla.

Fallik E., Sarig S. and Okon Y. (1994) Morphology and physiology of plant roots associated with *Azospirillum*. In *Azospirillum--Plant Associations* (Y. Okon, Ed.), pp. 77–86. CRC Press, Boca Raton, Fla.

Favilli F., Balloni W., Cappellini A., Granchi L. and Savoini G. (1987) Esperienze pluriennali di batterizzazione in campo con *Azospirillum* spp di coltore cerealicole. *Annals of Microbiology* **37**, 169–181.

Gillis M. and Reinhold-Hurek B. (1994) Taxonomy of *Azospirillum*. In *Azospirillum–Plant Associations* (Y. Okon, Ed.), pp. 1–14. CRC Press, Boca Raton, Fla.

Halliday J. (1984) Principles of *Rhizobium* strain selection. In *Biological Nitrogen Fixation, Ecology, Technology and Physiology*, (M. Alexander, Ed.), pp. 155–171. Plenum Press, New York.

Hartmann A. and Zimmer W. (1994) Physiology of *Azospirillum*. In *Azospirillum–Plant Associations* (Y. Okon, Ed.), pp. 15–39. CRC Press, Boca Raton, Fla.

Mertens T. and Hess D. (1984) Yield increases in spring wheat (*Triticum aestivum*) inoculated with *Azospirillum* lipoferum under greenhouse and field conditions of a temperate region. *Plant and Soil* **82**, 87–99.

Michiels K., Vanderleyden J. and Elmerich C. (1994) Genetics and molecular biology of *Azospirillum*. In *Azospirillum–Plant Associations* (Y. Okon, Ed.), pp. 41–56. CRC Press, Boca Raton, Fla.

Okon Y. (1985) *Azospirillum* as a potential inoculant for agriculture. *Trends in Biotechnology* **3**, 223–228.

Okon Y. (Ed.) (1994) *Azospirillum–Plant Associations*. CRC Press, Boca Raton, Fla.

Okon Y. and Kapulnik Y. (1986) Development and function of *Azospirillum*-inoculated roots. *Plant and Soil* **90**, 3–16.

Okon Y., Kapulnik Y. and Sarig S. (1988) Field inoculation studies with *Azospirillum* in Israel. In *Biological Nitrogen Fixation Recent Developments* (N. S. Subba Rao, Ed.), pp. 175–195. Oxford and I.B.H. Publishing Co., New Delhi.

Olubayi O., Ford T. M. and Neyra-Estens C. A. (1992) Growth response of several grass-species to inoculation with *Azospirillum brasilense* strain Cd. In *Abstracts of the 9th International Congress on Nitrogen Fixation*, Cancun, Mexico, 6–11 December, Abstr. No. 858.

Omar N., Heulin Th., Weinhard P., Alaa El-Din M. N. and Balandreau J. (1989) Field inoculation of rice with *in vitro* selected plant/growth promoting-rhizobacteria. *Agronomie* **9**, 803–808.

Paredes-Cardona E., Carcano-Montiel M., Mascarua-Esparza M. A. and Caballero-Mellado J. (1988) Respuesta del Maiz a la inoculacion con *Azospirillum* brasilense. *Revista Latinoamericana de Microbiologia* **30**, 351–355.

Rao N. S. S. (1986) Cereal nitrogen fixation research under the BNF coordinated project of the ICAR. In *Proceedings of the Working Group Meeting on Cereal Nitrogen Fixation* (S. P. Wani, Ed.), pp. 23–35. ICRISAT, Patancheru, India.

Reynders L. and Vlassak K. (1982) Use of *Azospirillum brasilense* as biofertilizer in intensive wheat cropping. *Plant and Soil* **66**, 217–223.

Rodriguez-Caceres E. A., Gonzalez-Anta G., Lopez J. R., Di Ciocco C., Pacheco-Basurco J. C. and Parada J. L. (1994) *Azospirillum brasilense* and *Bacillus polymyxa* inoculation in yield response of field grown wheat in an Argentine semiarid region. *Arid Soil Research and Rehabilitation*. In press.

Sadasivan L. and Neyra C. A. (1985) Cyst of Azospirilla under various cultural conditions In *Azospirillum III: Genetics, Physiology, Ecology* (W. Klingmuller, Ed.), pp. 230–242. Springer-Verlag, Berlin.

Sarig S., Kapulnik Y., Nur I. and Okon Y. (1984) Response of non-irrigated *Sorghum bicolor* to *Azospirillum* inoculation. *Experimental Agriculture* **20**, 59–66.

Sarig S., Blum A. and Okon Y. (1988) Improvement of the water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*. *Journal of Agricultural Sciences, Cambridge* **110**, 271–277.

Smith R. L., Schank S. C., Bouton J. R. and Quesenberry K. H. (1978) Yield increases in tropical grasses after inoculation with *Spirillum lipoferum*. *Ecological Bulletin (Stockholm)* **26,** 380–385.

Smith R. L., Schank S. C., Milam J. R. and Baltensperger A. A. (1984) Responses of *Sorghum* and *Pennisetum* species to the $N_2$-fixing bacterium *Azospirillum brasilense*. *Applied and Environmental Microbiology* **47,** 1331–1336.

Sumner M. E. (1990) Crop responses to *Azospirillum* inoculation. *Advances in Soil Sciences* **12,** 53–123.

Tien T. M. Gaskins M. H. and Hubbel D. H. (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* **37,** 1016–1024.

Vasuvat Y., Fangcham B., Siripin S., Fusang D. and Chan-a-ram P. (1986) Yield maximization of feed grain by associative $N_2$-fixing bacteria. In *Proceedings of an International Seminar on Yield Maximization of Feed Grains Through Soil and Fertilizer Management*, Bangkok, Thailand, 12–16 May.

Wani S. P. (Ed.) (1986) Cereal nitrogen fixation. In *Proceedings of the Working Group Meeting on Cereal Nitrogen Fixation*, p. 120. ICRISAT, Patancheru, India.

Wani S. P. (1990) Inoculation with associative nitrogen-fixing bacteria: role in cereal grain production improvement. *Indian Journal of Microbiology* **30,** 363–393.

Warenbourg F. R., Dressen R., Vlassak K. and Lafont F. (1987) Peculiar effect of *Azospirillum* inoculation on growth and nitrogen balance of winter wheat (*Triticum aestivum*). *Biology and Fertility of Soils* **4,** 55–59.

Zaady E., Okon Y. and Perevolotsky A. (1994) Growth response of mediterranean herbaceous swards to inoculation with *Azospirillum brasilense*. *Journal of Range Management* **47,** 12–15.

## APPENDIX

*List of participants*

**Carlos Labandera,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Salvador Cvrbelo,** Laboratorie de Microbiologia de Svelos, Comision Honoraria del lan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Pablo Dutto*,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Marta Rolfo,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Alicia Milian,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **David Pastorini,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Elena Beyhaut,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Elena Perez-Faggiani,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Francisco Canzani,** Laboratorio de Microbiologia de Suelos, Comision Honoraria del Plan Agropecuario, Ministerio de Ganaderia, Agricultura y Pesca, Burgues 3208, Montevideo, Uruguay. **Yaacov Okon,** Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel. **Guillermo Arrospide*,** Calister S.A., Juan Ramon Gomez, Montevideo, Uruguay. **Anibal Alvarez,** Enzur S.A., Azara, Montevideo, Uruguay. **Amalia Baraibar,** Lage y Cia. S.A., Camino Carrasco 6948, Montevideo, Uruguay. **Gonzalo Castro-Videla,** Agrobiotec S.A., Av. de Mayo 666 p.3, 1084 Buenos Aires, Argentina. **Enrique Rodriguez-Caceres,** Instituto de Microbiologia y Zooolgia, Agricola-CICA-INTA, C.C. 25, 1712 Castelar, Buenos Aires, Argentina. **Cesar Di Ciocco,** Catedra de Ecologia, Universidad Nacional de Lujan, Cruce Ruta 5 y 7, Lujan, Argentina. **Carlos Barassi*,** Area Biomolecular, Universidad Nacional de Mar del Plata, C.C. 276 INTA, 7620 Balcarce, Argentina. **Graciela Pozzo-Ardizzi*,** Universidad Nacional del Comahue, Casilla de Correo 149, 8500 Viedma, Rio Negro, Argentina. **Gabriela Aschkar,** Universidad Nacional del Comahue, Casilla de Correo 149, 8500 Viedma, Rio Negro, Argentina. **Graciela Pellejero,** Universidad Nacional del Comahue, Casilla de Correo 149, 8500 Viedma, Rio Negro, Argentina. **Mariza Abrameto,** Universidad Nacional del Comahue, Casilla de Correo 149, 8500 Viedma, Rio Negro, Argentina. **Azucena Monzon,** Catedra de Microbiologia, Facultad de Agronomia—U.B.A., Av. San Martin 4453, 1407 Capital Federal, Argentina. **Ines Garcia,** Catedra de Cereales, Facultad de Agronomia—U.B.A., Av. San Martin 4453, 1407 Capital Federal, Argentina. **Carlos Bellone and Silvia Carrizo,** Facultad de Agronomia y Zootecnia, Silvia Carrizo, Universidad Nacional de Tucuman, Av. Roca 1900, 4000 San Miguel de Tucuman, Argentina. **Liliana Goni,** Facultad de Agronomia, Universidad Nacional de La Pampa, Ruta 35 Km. 334, C.C. 300, 6300 Santa Rosa, La Pampa, Argentina. **Gloria Martinez-Drets,** Division Bioquimica, Inst. Invest. Biol. Clemente Estable, Av. Italia 3318, Montevideo, Uruguay. **Lillian Frioni,** Catedra de Microbiologia, Facultad de Agronomia, Garzon 791, Montevideo, Uruguay. **Gustavo de los Santos,** Catedra de Micnrobiologia, Facultad de Agronomia, Garzon 791, Montevideo, Uruguay. **Luis Acuna,** INSUR, Sarmiento 2404 esc. 404, 11300 Montevideo, Uruguay.

*Discussion panel coordinators.