There is a definite need for the identification of the most critical parameters for beneficial association.

In the discussion to follow, the successes and failures of *Azospirillum* inoculation in relation to N_2 fixation and hormonal and other effects will be analyzed. The literature will be reviewed from the agronomic perspective so that a conclusion may be reached as to whether or not *Azospirillum* inoculation will become a feasible and profitable technique in the near future.

Before proceeding to an evaluation of greenhouse and field growth responses, it is necessary to review briefly the interaction of *Azospirillum* with roots. In this manner, a better understanding of growth responses and the resultant morphological and chemical changes may be obtained.

II. Interactions of *Azospirillum* with Roots

A. *Azospirillum* spp.

Azospirillum is ubiquitous in the rhizosphere of grasses in the field. Usually two species are found, *A. lipoferum*, most commonly as a surface-sterile isolate from the roots of C-4 grasses, and *A. brasilense* from C-3 grasses and sugarcane (C-4) in tropical and subtropical regions. However, Horemans et al. (1988) have recently found no evidence for the presence of *Azospirillum* within the roots of barley, wheat, corn, or grasses grown under field conditions in Belgium, with *A. lipoferum* being found only on the root surfaces of corn and grass and *A. brasilense* on the roots of all crops investigated. Concentrations of these bacteria never exceeded 10^3 colony-forming units (cfu) per gram of soil. *Azospirillum* is microaerophilic and shows a marked preference for low p_{O_2} with very little nitrogenase activity at p_{O_2} values above 1 kPa (Patriquin et al., 1983). The main difference between the two species is the ability of *A. lipoferum* to produce acid in the fermentation of sugars, whereas *A. brasilense* possesses primarily an oxidative metabolism. Under microaerophilic conditions *Azospirillum* fixes N_2, but under aerobic conditions it is capable of utilizing combined inorganic and organic forms of N. Several strains are capable of denitrification, which is favored under the same conditions as those that promote nitrogenase activity (Danneberg et al., 1985; Hubbell and Gaskins, 1984).

B. Adsorption to Plant Roots

Because plant roots excrete organic and inorganic substances suitable as nutritional sources for microorganisms, it is not surprising to find elevated numbers of bacteria in the rhizosphere of plants. On the one hand, in highly specific systems, such as the *Rhizobium*–legume association, large numbers of bacteria are selectively adsorbed on the root hairs of the homologous host, where they can be weakly or strongly attached, with the latter competing better in colonization and nodulation (Vance, 1983). On the other hand, in experiments with nonspecific bacteria adsorption did not follow the Langmuir adsorption isotherm, whereas with *Rhizobium*, proper isotherms were obtained and the adsorption appeared to be via a specific protein (Shimshick and Herbert, 1979).

In the case of *A. brasilense* strain Sp 7, polar adsorption to root hairs and old epidermal cells was rapid, with age of culture and suspending electrolyte affecting the number of bacteria adsorbed. After several hours, fibrillar structures appeared as anchors to the root. Azospirilla were firmly attached to root hairs of grasses grown in the absence but not in the presence of fixed nitrogen, in which case they were associated only with the mucigel at the root cap and with undifferentiated epidermal cells. Significantly fewer azospirilla attached to millet roots when grown in media containing 5 m*M* potassium nitrate (Umali-Garcia et al., 1980). Several workers (Kapulnik et al., 1985a; Umali-Garcia et al., 1980, 1981) showed that the adsorption of different strains of *A. brasilense* to wheat *(Triticum aestivum)*, pearl millet *(Pennisetum americanum)*, and guinea grass *(Panicum maximum)* was greatly affected by the growth phase of the culture. The attachment properties of different *Azospirillum* strains on wheat roots suggests that they are affected at the bacterial genome level (Jain and Patriquin, 1984). Presumably, mechanisms by which *Azospirillum* attaches to root hairs and undifferentiated epidermal cells differ in response to changes caused by fixed nitrogen in the rooting medium (Patriquin et al., 1983; Umali-Garcia et al., 1980, 1981). More recently, adsorption of *A. brasilense* strain Cd on corn *(Zea mays)* roots increased rapidly for 90 minutes, reaching a maximum after 4.5 hours at a pH of 6.1, with other strains being adsorbed to different extents, each having its own optimum pH value (Okon and Kapulnik, 1986). Bacterial adsorption occurred on cells in the root elongation zone and at the bases of root hairs and followed the Langmuir isotherm up to 10^9 cells/mL, above which larger numbers than expected were adsorbed, probably due to the adsorption of aggregates of cells. Binding of *A. brasilense* strain Cd was reduced in the presence of Ca, Mg, Na, poly (L-lysine), several sugars, and amino and organic acids at pH 6.1 with evidence of bacterial response to chemotactic gradients. This possibly provides the bacteria with the ability to reach and preferentially colonize plant roots. Hess et al. (1985) demonstrated that sucrose, glucose, and fructose attracted azospirilla to wheat roots with the chemotactic potential being increased by sucrose cleavage by an invertase secreted by the roots. Azospirilla adhered to pearl millet roots in greater numbers than all other bacteria examined. This indicates that grass root hairs exhibit selectivity in binding bacteria (Table 1). *R. trifolii* and *P. fluorescens* also adhere to millet roots and, interestingly, they share certain serological and biochemical characteristics with *Azospirillum*. Adsorption of *R. japonicum* to wheat and rice *(Oryza sativa)* roots has been reported previously (Shimshick and Herbert, 1979). Preincubation of *A. brasilense* with root exudates promoted adsorption. This effect was negated by treatment of the extracts with enzymes (protease and trypsin), which suggests the possible involvement of proteins-lectins in the adsorption–recognition process of *Azospirillum* to grass roots. Indeed, *A. lipoferum* strains 4B and A-95 stimulated root exudation in rice plants, whereas strains RO7 and B7C did not (Heulin et al., 1987). No information is available on the effect of random or specific adsorption on colonization and proliferation on and in the roots (Okon and Kapulnik, 1986), but Eyers et al. (1988) have shown that live *A. brasilense* strains Sp 7 and Sp 245 attach to plant cells more strongly than to dead cells.

Table 1. Adsorption of various bacteria to root hairs of pearl millet *(Pennisetum americanum)* seedlings

Bacterium root	Number of root hairs examined	Number of adsorbed cells per hair
Azospirillum brasilense strain Sp 13t	16	23.7 ± 2.4
Azospirillum brasilense strain Sp 7	18	33.0 ± 4.0
Azospirillum brasilense strain JM 125A2	19	32.5 ± 2.9
Rhizobium trifolii strain 0403	19	14.4 ± 2.0
Pseudomonas fluorescens	15	11.4 ± 2.9
Azotobater vinelandii strain UW10	15	0.6 ± 0.3
Klebsiella pneumoniae	18	0.3 ± 0.2
Escherichia coli	16	0.1 ± 0.1

From Umali-Garcia et al. (1980).

C. Root Colonization

It is well known that in the *Rhizobium*–legume association and with root pathogens there is considerable specificity in the infection of the host, which is also likely to be the case in *Azospirillum*–gramineae associations.

In order to obtain yield responses to field inoculation with *Azospirillum*, it is essential that the strain or strains used establish themselves and multiply in the rhizosphere or in roots where indigenous strains and other organisms offer stiff competition. The enrichment of the rhizosphere in *Azospirillum* relative to the bulk soil can be brought about by any one or a combination of the following: (1) competitive advantage in the carbon-rich, nitrogen-poor environment, (2) supply by the plant of essential vitamins, (3) aerotactic attraction to reduced p_{O_2} in the root zone (Patriquin et al., 1983), and (4) chemotactic attraction to root exudates and organic acids (Baldani et al., 1986). In general, while bacteria are randomly distributed on grass roots, *Azospirillum* colonization usually occurs on mucigel-covered epidermis, in the elongation zone, on the bases of root hairs, and where root branches emerge. Bacteria were found in the mucigel that accumulated on the root caps and along the root axes, but fewer bacteria were present on the root cap or adsorbed to root hairs of wheat (Kapulnik et al., 1985a). The viscous nature of mucigel would provide a barrier to gas diffusion, which may create a microaerophilic environment conducive to N_2 fixation. Adherent bacteria were associated with granular material on root hairs and fibrillar material on undifferentiated epidermal cells (Okon and Kapulnik, 1986; Umali-Garcia et al., 1980). Tilak and Subba Rao (1987) found that *Azospirillum* occurred as encrustations in the mucilage, colonizing root hairs and the innermost layer of cortex, and conducting vessels in pearl millet with almost total absence toward the root tip. Relatively large populations of *Azospirillum*, contributing significantly to microbial biomass, are now known to invade root interiors, where they reside between cortical cells and in apparently dead cells (Baldani and Dobereiner, 1980; Patriquin et al., 1983; Umali-Garcia et al., 1980), although Reinhold et al.,

(1986) found evidence of close association between *A. lipoferum* and a new type of *Azospirillum* spp. and kallar grass *(Leptochloa fusca)* on the rhizoplane with unidentified diazotrophic straight rods in the endorhizosphere. This distribution of the populations was stable, suggesting that different diazotrophs may be adapted to colonize different root zones of their hosts. Bashan and Levanony (1988) showed that after inoculation of wheat with *A. brasilense* strain Cd, small aggregates of bacteria formed on the root surface, with a larger population within the cortex and no bacteria in the xylem. A few bacteria penetrated young live root cells, but the majority occurred in intercellular spaces or adsorbed to cortical or epidermal cell walls by an electron-dense material. As a result of the inoculation, proton exudation and cell division at the root tip were increased. As *Azospirillum* has pectinolytic activity (see later), it is not surprising that it can penetrate root hairs and other cells. However, no evidence of the formation of viable, intracellular associations with grasses has been found. The importance of these azospirilla within the root is indicated by the fact that in wheat, total N accumulation was correlated with numbers recovered from surface-sterilized but not untreated roots (Baldani et al., 1983). In rice inoculated with *A. lipoferum* strain 34-H, the root tips of one cultivar (IR42) were not initially colonized, whereas those of another (IR50) were. In the early stages, most of the bacteria were embedded in the ruptured mucigel below the root cap cells of IR42, while mucigel was hardly detectable in IR50 (Murty and Ladha, 1987). The root hair primordia of IR50 were more rapidly colonized than those of IR42. Thus adherence of bacteria may depend on the nature of the root surface rather than on the presence or absence of mucigel. It was suggested that *Azospirillum* invades the intercellular spaces in the inner cortex and stele and the lumen of the xylem vessels without disrupting the outermost cortex or endodermis. It appears that the initial entry into the stele occurs at apical regions where the endodermal walls and Casparian strip have not thickened and where the stele has not differentiated. This assumes that the normal processes which resist bacterial invasion are not operative at the root tip, which was the site of initial colonization of soybean *(Glycine max)* roots. This internal association between *Azospirillum* and the host would facilitate an efficient exchange of materials, and the presence of substrate and low p_{O_2} in the xylem would be conducive to N_2 fixation, but the low pH of the xylem sap would tend to prevent this process (Patriquin et al., 1983).

While there are no observations suggesting that N_2 fixation or yield is related to any specific sites of *Azospirillum* colonization, substantial evidence exists to suggest some internal localization or protective structure:

1. Microbial populations obtained from surface-sterilized roots are different from untreated roots, with the former being enriched in azospirilla. No consistent predominance of either *Azospirillum* species occurred when the bacteria were isolated from washed roots of rice, wheat, or maize. However, after surface sterilization with chloramine-*t*, *A. lipoferum* was dominant in isolates from maize and other C-4 graminaceous crops (except sugarcane [*Saccharum officinarum*]) and *A. brasilense* nir⁻ from rice, wheat, and other C-3 crops (Baldani and dobereiner, 1980).

2. Intact cortex and increased root activity with increasing lateral roots are consistent with colonization through root tips (Dobereiner and Day, 1976).
3. High apparent rate constants for acetylene reduction activity (ARA) and postlag ARA insensitivity to p_{O_2} are consistent with the presence of barriers to gaseous diffusion to and from N_2-fixing sites (Patriquin, 1978).
4. Total numbers of *Azospirillum* within the root are correlated with total N accumulation, which is not the case with non-surface-sterilized roots (Baldani et al., 1983).
5. N_2 fixation is low in leaky and high in nonleaky roots of wheat lines (Rennie and Larson, 1979).
6. "C forms," spheres, and other structures formed by *Azospirillum* are bound by a membrane to the root surfaces of wheat (Patriquin et al., 1983). This would afford some degree of protection from external oxygen.
7. The effect of metabolic poisons applied to the root surface is consistent with the internal localization of some N_2-fixing sites (Boyle and Patriquin, 1980).
8. Direct evidence from electron microscopy shows internal colonization by a limited number of bacteria, with a zone relatively free of bacteria separating the majority of the bacteria from the root surface (Whallon et al., 1985).

Reinhold et al. (1986) suggested that, since large numbers of N_2-fixing bacteria occur within the root, which may result in an advantage for substrate competition against nondiazotrophs, endorhizosphere bacteria might be more important for N supply to the host than rhizoplane bacteria. However, no quantitative estimates of *Azospirillum* biomass on or in the roots are available (Okon et al., 1988).

The effect of encapsulation on the attachment process is not well understood, but *Azospirillum* forms various structures like *Rhizobium*, which are thought to confer oxygen protection and resistance to desiccation (Patriquin et al., 1983). However, none of these structures are visible to the eye, and thus successful infection cannot be evaluated easily. When incubated for prolonged periods, *Azospirillum* produces cystlike ovoids, which may provide a mechanism for survival under adverse conditions in the rhizosphere (Hubbell and Gadkins, 1984). Sadasivan and Neyra (1985) demonstrated the formation of desiccation-resistant cysts in *A. brasilense* strain Sp 7 and *A. lipoferum* strain Sp 59b associated with brown-black melaninlike granular pigments under nitrogen-limiting conditions. The formation of these cysts appears to be controlled by genetic and environmental factors.

Thus plant roots respond in different ways to varying levels and kinds of azospirilla, but, in general, the effect seems similar to the symptoms obtained when plants are infected with varying levels of pathogenic bacteria. There is evidence that a recognition process is involved in colonization similar to the lectin-recongition model in the *Rhizobium*–legume association (Umali-Garcia et al., 1980). Colonization probably takes place at specific sites, favored by an optimal inoculum level in each case. In addition, there is probably substantial competition for sites by other rhizosphere organisms. Furthermore, evidence

indicates that nitrate and, to a lesser extent, ammonium strongly inhibit attachment of nonhomologous but not homologous strains to maize roots.

Schmidt et al. (1988) showed that inoculation of alfalfa *(Medicago sativa)* with a combination of *Rhizobium meliloti* and *A. brasilense* strain Cd in sterile agar resulted in a significant increase in the number of nodules depending on the concentration of *Azospirillum*. The stimulating effect is probably due to the production of indol-3-acetic acid (IAA), which in pure form leads to increased nodulation. An increase in nodule number was also found with soybeans grown in soil under nonsterile conditions upon repeated inoculation with *Azospirillum*. *Azospirillum brasilense* was much more effective in producing IAA than *A. lipoferum*. Yahalom et al. (1987), working with pouch-grown *Medicago polymorpha* and *Macroptilium atropurpureum* seedlings, showed the preinoculation with *A. brasilense* strain Cd 24 hours before Rhizobium resulted in increased nodulation and ARA. The stimulation of a larger number of epidermal cells that differentiate into infectable roots by the *Azospirillum* inoculation was advanced as a possible reason for the increased susceptibility to *Rhizobium* infection. Similar results were obtained with various forage legumes grown in pot culture (Yahalom et al., 1988). On the other hand, Raverkar and Konde (1988) found that inoculation of peanuts *(Arachis hypogaea)* with *A. lipoferum* and *Rhizobium* strain NC92 singly increased nodule number, nodule dry weight, amount of dry matter produced, and N content, but inoculation with a mixture of these organisms largely negated the effect on pod yield and decreased it with respect to the other parameters.

D. Establishment and Proliferation in the Field

Relatively little information has been published on the establishment and multiplication of *Azospirillum*, especially under field conditions. Following inoculation, many strains survive in the rhizosphere, but invariably populations stabilize at low levels. Albrecht et al. (1983) and Smith et al. (1984b) reported that *A. brasilense* strains 13t SR2 and CdSR failed to establish in the rhizosphere of sorghum *(Sorghum bicolor)*, maize, and pearl millet in Florida. In extensive field experimentation in Brazil, Baldani et al. (1986) demonstrated that *A. brasilense* strains Sp 107 and Sp 245 could be established in all wheat experiments and were the dominant strains in washed and especially surface-sterilized roots, whereas strains Sp 7 and Cd established poorly. *Azospirillum lipoferum* strain Sp S82 was the dominant root isolate from sorghum inoculated with this strain, which became concentrated in the upper parts of the root system. On the other hand, Harris et al. (1989) showed that *Azospirillum* is not a good colonizer of wheat rhizospheres after inoculation under temperate conditions. Thus no physical or chemical treatments appear to bring about the preferential increase in *Azospirillum* numbers in soil. Whether this will ever become possible is not clear, but a more likely possibility would involve specificity between bacterium and host that would provide *Azospirillum* with a competitive edge. However, there still remain many technical problems to be overcome before proper colonization of roots can

be assured under a variety of soil and environmental conditions. The use of pesticides may have an effect on *Azospirillum* performance, but to date no field studies have been carried out. Herbicides such as metrabuzin and ethiozin have short-term inhibitory effects on the nitrogenase activity of *A. brasilense* and *A. lipoferum* under laboratory conditions, but the effects under field conditions are unknown (Gadkari, 1988). The organochloride insecticide Difocol at concentrations less than 1 ppm under laboratory conditions has been shown to stimulate the nitrogenase activity of *A. lipoferum* strain Sp Br17, but above 10 ppm cell viability and nitrogenase activity were severely reduced, resulting in the formation of cystlike structures which may reflect an induced resistance mechanism (Mano et al., 1988).

E. *Azospirillum* and Root Morphology and Function

1. Root Hair Deformation

Inoculation with *Azospirillum* spp. has a profound effect on root morphology and function. Root hair numbers and differential root lengths are increased, with individual strains showing effects of differing intensity. Basically two types of branching, namely, "tuning fork deformation" with branches of equal length and branches of unequal length, were observed in field-grown wheat seedlings inoculated with *A. brasilense*, with strain Sp 245 being more effective than strains Sp 7 and Sp 107. High frequencies of deformed wheat and maize root hairs were observed in semisolid agar but not in water agar (Patriquin et al., 1983; Tien et al., 1979). In semisolid and water agar, *Azospirillum* seems to promote tuning fork deformations with the effect in *A. brasilense* strain Sp 245 > strain Sp 107 > strain Sp 7 > *Azospirillum* spp. strain Sp 242, which corresponds to the order of total N accumulation in wheat following inoculation of field-grown wheat (Baldani et al., 1983). Few or no tuning fork deformations are produced by nonhomologous strains Sp 7 and Sp 242. This suggests that tuning fork deformations may have some prognostic value. The number of deformations was higher in the presence than in the absence of N, which is contrary to the effect in legumes. Root hair deformations were also observed in field-grown maize, but deformations could not be induced in agar cultures, even with homologous strains of *Azospirillum*. If hydrolytic enzymes are responsible for root hair deformations in wheat, as the lectin-enzyme hypothesis of Hubbell (1981) would suggest, then root hair deformation may be related to the ability of strains to invade the root. This is supported by the observations of a high degree of activity present in populations isolated from surface-sterilized but not from untreated roots (Baldani and Dobereiner, 1980; Baldani et al., 1983; Patriquin et al., 1983). In a number of crop plants, *Azospirillum* inoculation at 10^5 cfu/mL resulted in denser and longer roots hairs (2.5 cm from the root cap) than in controls treated with dead cells. This did not affect the length of the elongation zone, which suggests that the effect is due to an earlier initiation of root hair formation. At 10^8 cfu/mL, the elongation zone length was greatly decreased with

severe deformation of the root cap. In *Sorghum vulgare* × *Sorghum sudanense* inoculated with *A. brasilense* strain Cd, the distance between root cap and hairs was significantly greater than in untreated controls at 10^5 cfu/mL, but it was significantly smaller at 10^8 cfu/mL (Okon and Kapulnik, 1986).

In general, effects on root hair development are more marked at high inoculum concentrations, which might explain why root hair deformations were observed in semisolid agar, which would favor bacterial proliferation, but not in water agar (Patriquin et al., 1983). It is not clear whether the effects on root hair formation are caused by root cap growth inhibition effects on cell division or by elongation at the elongation zone, or whether increased root hair formation is caused by the differentiation of cells in the elongation zone to form root hairs. All these effects depend on and vary with inoculum concentration, bacterial and plant type, method of growing the plant, and environmental conditions (Okon and Kapulnik, 1986).

2. Root Elongation and Surface Area

Root elongation was increased in a number of crops (*Pennisetum, Setaria italica,* and wheat) by *Azospirillum* inoculation under both greenhouse and field conditions. With a single wheat cultivar at 10^5 to 10^6 *Azospirillum* cfu/mL, both root length (Figure 1) and total surface area reached a maximum (Kapulnik and Okon, 1983), and the magnitude of the effect varied according to culture age (lag phase cultures being less effective) and incubation temperature, but 10^8 to 10^{10} cfu/mL inhibited root development. With a mixture of *Azospirillum* strains and a variety of wheat cultivars, inoculation caused an increase in root length in all cultivars, but increased surface area only in some (Figure 2). Other bacteria in the genera *Klebsiella, Azotobacter, Bacillus,* and *Pseudomonas* did not increase the surface area of wheat roots (Kapulnik and Okon, 1983; Okon and Kapulnik, 1986: Tien

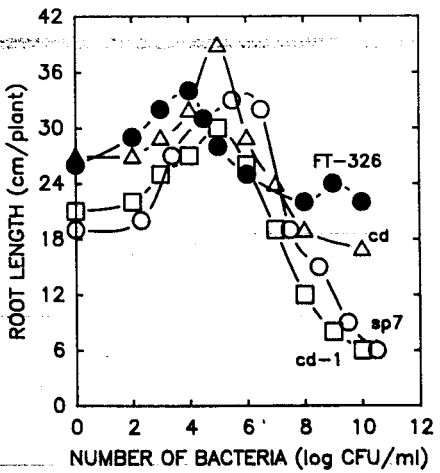

Figure 1. Effect of inoculum concentration of different strains of *Azospirillum* on root elongation of wheat seedlings cultivar Miriam. • FT-326, IAA overproducing mutant; △ Cd; ○ Sp 7; □ Cd-1. [From Kapulnik et al. (1985a).]

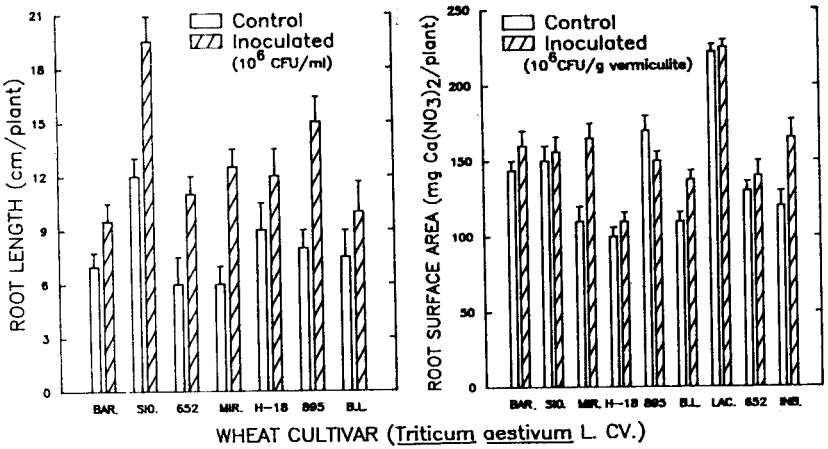

Figure 2. Effect of *Azospirillum*-mixed inoculum on root length and surface area of different wheat cultivars. [From Kapulnik et al. (1985a).]

et al., 1979; Yahalom et al., 1984) (Table 2). In C-3 (barley [*Hordeum vulgare*] and wheat) and C-4 (*Panicum miliaceum*) grasses, different and mixed strains of *Azospirillum* increased the elongation of seminal roots with bacterial concentration. Elongation was optimal in the range of 10^3 to 10^6 cfu/mL, above which roots were inhibited. Optimal bacterial concentrations and critical levels for inhibition varied for the different species and strains tested (Okon and Kapulnik, 1986). These data suggest that *Azospirillum* behaves in a manner similar to phytopathogenic bacteria because of the relationship between bacterial concentration and development of symptoms. As specific sites probably exist on roots where colonization is initiated, there is likely to be an optimal inoculum concentration for eliciting positive responses in root growth and development. Because the optimal inoculum level will depend on *Azospirillum* strain, plant type, and environmental conditions, these factors must be considered in developing inoculants and application methods for introducing the required number of appropriate root-colonizing bacteria for different types of soil.

III. Yield Responses to *Azospirillum* Inoculation

A. Greenhouse and Laboratory

A summary of the yield responses to *Azospirillum* of many crops published in the literature is presented in Table 3 with relevant information on N content, N uptake, and ARA. The responses fall into the following broad categories: (1) those where only yield increases without pertinent measures of N_2 fixation were

Table 2. Comparison of the effect of *Azospirillum* and other bacteria on wheat root development

Bacterium	Concentration (cfu/mL)	Increase in root length (%)	Increase in root surface area (%)
Klebsiella pneumoniae	10^6	6.8 ns[a]	18.2 ns
	10^8	6.8 ns	15.8 ns
Azotobater chroococcum	10^6	27.6 *[b]	–4.0 ns
	10^8	20.6 *	–4.0 ns
Azospirillum	10^6	20.6 *	33.7 *
	10^8	1.3 ns	2.1 ns
Bacillus megaterium	10^6	13.8 ns	–5.4 ns
	10^8	2.7 ns	–5.8 ns
Pseudomonas syringae	10^6	6.9 ns	6.7 ns
	10^8	–11.0 ns	10.3 ns
Control (dead *Azospirillum* cells)		—	—

From Kapulnik and Okon (1983).

[a]ns = No significant difference.

[b]* = Significant at the 0.05 probability level.

recorded [Jagnow (1985) with *Arrhenatherum elatius* and *Festuca rubra;* Kapulnik et al. (1981c, 1985c) with *Setaria italica* and *Triticum aestivum* [roots only]; Lin et al. (1983) with *Zea mays* and *Sorghum bicolor;* Millet et al. (1985) with *Triticum aestivum* and *Triticum turgidum;* Nayak et al. (1986) with *Oryza sativa;* Tien et al. (1979) with *Pennisetum americanum;* and Subba Rao et al. (1979) with *Oryza sativa]*, (2) those where no yield responses were obtained to inoculation [Albrecht et al. (1981) with *Zea mays;* Baltensperger et al. (1978) with *Cynodon dactylon;* Bouton and Zuberer (1979) with *Panicum maximum;* Haahtela et al. (1988) with *Poa pratensis* and *Triticum aestivum;* and Schank et al. (1981) with *Digitaria* spp.], and (3) those where positive yield responses and increased N uptake, or increased ARA, or both, have been observed [Avivi and Feldman (1982) with *Triticum durum;* Bouton and Zuberer, (1979) with *Panicum maximum;* Cohen et al. (1980) with *Setaria italica* and *Zea mays;* Fayez and Daw (1987) with *Gossypium barbadense;* Fayez et al. (1985) with wheat; Hadas and Okon (1988); Hegazi et al. (1983) with *Zea mays;* Kapulnik et al. (1981a, 1981b) with *Panicum miliaceum, Sorghum bicolor* × *Sorghum sudanense, Sorghum bicolor,* and *Setaria italica;* Lethbridge and Davidson (1983) with *Triticum aestivum;* Malik et al. (1987) with *Leptochloa fusca;* Mertens and Hess (1984) with *Triticum aestivum;* Nayak et al. (1986) with *Oryza sativa;* Nur et al. (1980) with *Zea mays* and *Setaria italica;* O'Hara et al. (1981) with *Zea mays;* Rai and Gaur (1988) with *Triticum aestivum;* Rennie (1980) with *Zea mays;* Rennie and Larson (1979) with *Triticum aestivum* (disomic chromosome substitution lines); Smith et al. (1976) with *Digitaria decumbens;* Ven-

kateswarlu and Rao (1983) with *Pennisetum americanum;* Yahalom et al. (1984) with *Setaria italica;* and Watanabe and Lin (1984) with *Oryza sativa]*. Where N uptake or ARA was measured, no cases were observed where inoculation caused a decrease in either of these values. There are cases where specific strains are required to elicit a yield response [Cohen et al. (1980) with *Zea mays* and *Setaria italica;* Fayez and Daw (1987) with *Gossypium barbadense;* Ferreira et al. (1987) with *Triticum aestivum;* Lin et al. (1983) with *Sorghum bicolor;* O'Hara et al. (1981) with *Zea mays;* O'Hara et al. (1987) with *Zea mays* and *Pennisetum americanum;* Rai and Gaur (1988) with *Triticum aestivum;* and Venkateswarlu and Rao (1983) with *Pennisetum americanum]*, pointing to the importance of inoculating crops with homologous strains of *Azospirillum*. In certain cases, increased root growth accompanied the yield response, but this was not consistent in all cases. From the results presented in Table 3, it can be safely concluded that, provided an appropriate inoculating strain is used, *Azospirillum* inoculation results in increased yields and N uptake for a wide range of tropical and temperate crops under a wide range of controlled conditions in the greenhouse and the laboratory. There is also substantial evidence that inoculation with *Azospirillum* increases rooting, as will be discussed later. It is pertinent to note that relatively few cases have been reported where no yield responses were obtained to inoculation. This does not necessarily imply that such cases do not exist.

B. Field

It is encouraging to discover that many workers have conducted field inoculation experiments with *Azospirillum*. This is relatively rare for such a recently discovered phenomenon. Results are summarized in Table 4 with pertinent data on N content, uptake, and fixation. These results fall into the following categories: (1) those where inoculation failed to elicit a yield response [Baldani et al. (1987) with *Triticum aestivum;* Marocco and Lorenzoni (1984) with *Triticum aestivum;* Millet et al. (1985) with *Triticum aestivum;* Taylor (1979) with *Panicum maximum;* Tilak and Subba Rao (1987) with *Pennisetum americanum;* Wani et al. (1985) with *Pennisetum americanum;* and Zambre et al. (1984) with *Triticum durum]*, (2) those where yield responses were obtained but no accompanying N data were presented [Bouton et al. (1979) with *Pennisetum americanum;* Charyulu et al. (1985) with *Oryza sativa;* Hegazi and Saleh (1985) with *Triticum aestivum;* Kannaiyan et al. (1983) with *Oryza sativa;* Kapulnik et al. (1983) with *Triticum aestivum;* Kapulnik et al. (1987) with *Triticum aestivum;* Marocco and Lorenzoni (1984) with *Hordeum vulgare;* Okon et al. (1988b) with *Zea mays, Triticum aestivum, Sorghum bicolor, Setaria italica,* and *Panicum miliaceum;* Rao et al. (1983) with *Oryza sativa;* Smith et al. (1976) with *Pennisetum americanum* and *Panicum maximum;* Taylor (1979) with *Pennisetum americanum;* and Tilak and Subba Rao; (1987) with *Pennisetum americanum]*,(3) those where positive yield responses were presented with supporting data [Albrecht et al. (1981) with *Zea mays;* Boddey et al. (1986) with *Triticum*

Table 3. Greenhouse yield responses of various crops to inoculation with *Azospirillum* spp. together with corresponding N content and uptake and acetylene reduction activity

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
			<		various units[a]					>	
Digitaria decumbens	Soil	*A. lipoferum* Sp 13t	No	0	180			1.90		1.06	Smith et al. (1976)
			Yes	0	300*			3.07		1.02	
Panicum maximum			No	0	800			11.01		1.38	
			Yes	0	1228*			20.05		1.63	
Cynodon dactylon	Soil	*A. brasil/ paspali*	Dead	0	5.40	3.05		40.50			Baltensperger et al. (1978)
			Live	0	5.63	2.88		49.00*			
Pennisetum americanum	Solution	*A. brasilense* Sp 13t SR2	No	0	52.67	12.01					Tien et al. (1979)
			Yes	0	74.47*[b]	12.05					
			No	21	694	161					
			Yes	21	1017*	211					
Panicum maximum	Soil	*A. brasilense* Sp 13t	Water	60	2.68		71				Bouton and Zuberer (1979)
			Live	60	3.50*		100				
			Filtrate	60	3.43		90				
			Water	40	2.10		96				
			Live	40	1.81		152				
			Filtrate	40	1.70		45				
Oryza sativa	Soil	*A. brasilense*	No	0	15.8						Subba Rao et al. (1979)
			Yes	0	20.9*						
			No	40	33.9						
			Yes	40	41.3*						
			No	60	48.1						
			Yes	60	54.4*						
			No	120	68.9						
			Yes	120	70.6						

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
			<----		various units[a]					---->	
Triticum aestivum	Solution	*A. brasilense*	No	100	287	101		14.88			Rennie and Larson (1979)
Cadet		Sp 7	Yes	100	436	172*		22.51*			
C-R2A			No	100	256	116		12.90			
			Yes	100	543*	185*		28.00*			
C-R2D			No	100	408	160		19.04			
			Yes	100	748*	136		32.60*			
C-R5B			No	100	389	77		14.77			
			Yes	100	413	98		19.03*			
C-R5D			No	100	320	124		15.11			
			Yes	100	405*	142		20.09*			
Rescue			No	100	372	134		20.14			
			Yes	100	349	156		18.11			
R-C2A			No	100	509	81		21.80			
			Yes	100	429*	146*		20.34			
R-C2D			No	100	339	167		17.12			
			Yes	100	603*	124		25.57*			
R-C5B			No	100	411	94		18.11			
			Yes	100	521*	193*		26.75*			
R-C5D			No	100	270	131		14.44			
			Yes	100	377*	384*		20.94*			

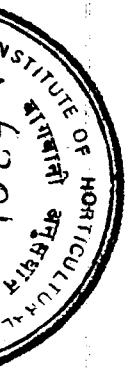

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
			<		various units[a]					>	
Zea mays	Soil	*A. brasilense*	No		1.470			10.23c		0.712	Nur et al.
		Cd	Yes		.759			14.08a		0.801	(1980)
		Sp 7	Yes		.538			11.24bc		0.731	
		Cd-1	Yes		.828			13.56a		0.742	
		Cd-3	Yes		.650			11.80bc		0.715	
Setaria italica			No		.577b	0.4500b	0	1.40b		0.906	
		Cd	Yes		2.821a	1.0020a	709	3.20a		1.127	
		Sp 7	Yes		2.569a	0.6842b	681	2.90ab		1.119	
		Cd-1	Yes		2.272ab	0.7856ab	585	2.50ab		1.084	
		Cd-3	Yes		2.175ab	0.5590b	557	2.10ab		0.988	
			No		1.4b		0	11.00b		0.80	
		Cd	Yes		2.5a		140	33.70a		1.33	
		Sp 7	Yes		2.4a		152	31.00a		1.23	
		Cd-1	Yes		2.2ab		140	20.00a		1.17	
		Cd-3	Yes		1.9ab		135	20.50ab		1.10	
Zea mays	Solution	*A. brasilense*	No	0	.59	0.27	0				Cohen et al.
		Sp 7	Yes	0	.85*	0.36	200				(1980)
			No	0.04	1.19	0.53	0				
			Yes	0.04	1.73*	0.76	156				
			No	0.08	2.09	0.86	0				
			Yes	0.08	2.81*	0.97	10				
			No	0.16	4.22	1.70	0				
			Yes	0.16	4.89	1.96	0				
			No	0.06	1.97	0.86	0				

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
			<		various units"					>	
		Sp 7	Yes	0.06	1.05*	1.40	101				
		Sp-80	Yes	0.06	1.22	1.01	80				
		Cd	Yes	0.06	1.84*	1.35	99				
		Cd-1	Yes	0.06	2.21	0.98	77				
Setaria italica	Solution	*A. brasilense*	No	0.06	1.62	0.45	0	10.50		0.9	
		Sp 7	Yes	0.06	2.82*	1.23	112	15.90*		1	
		Sp-80	Yes	0.06	2.22	0.83	98	13.90		1	
		Cd	Yes	0.06	2.14	0.85	88	12.90		1	
		Cd-1	Yes	0.06	1.93	0.75	80	11.80		1	
Zea mays			No	0.06	4.53	1.60	0	20.80		0.71	
		Sp 7	Yes	0.06	5.72*	2.14	351	26.77*		0.75	
		Cd-1	Yes	0.06	5.46*	2.02	410	25.24*		0.73	
Setaria italica			No	0.06	1.19	0.54	0	5.22		0.8	
		Sp 7	Yes	0.06	2.15*	0.90	426	11.41*		0.91	
		Cd-1	Yes	0.06	2.04*	0.83	378	11.21*		0.93	
	Sand		No	0	0.87	0.53	0	2.20		0.65	
		Sp 7	Yes	0	1.55*	0.84	902	5.50*		0.78	
	Sandy Loam		No	17	1.74	0.71	0	6.70		0.65	
			Yes	17	3.05*	1.51	1322	12.90*		0.84	
	Loess		No	340	4.83	1.53	0	34.20		1.06	
			Yes	340	6.05*	2.05	2050	47.00*		1.19	

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
				<-------- various units[a] -------->							
Zea mays	Solution	*A. brasilense*	No	0.26+ sucrose	310			1.39		0.46	Rennie (1980)
		Sp 7	Yes	0.26+ sucrose	403*			1.75*		0.43	
			No	0.26+ malate	333			1.55		0.46	
			Yes	0.26+ malate	470*			2.02*		0.43	
			No	0.26+ succinate	367			1.58		0.42	
			Yes	0.26+ succinate	580*			2.38*		0.41	
			No	0.26	388			2.11		0.55	
			Yes	0.26	453*			2.49*		0.55	
Zea mays	Soil	*A. brasilense*	No	0	12.4	2.52		49.4		0.4	Albrecht et al. (1981)
		Sp 7	Yes	0	11.3	3.67		59.9		0.52	
			No	15	15.7	4.27		156.0		0.99	
			Yes	15	14.7	4.68		140.0		0.95	
Digitaria sp	Clay	*A. brasilense* (mixed)	No	0	15.9	3.6	3.4	0.221		1.4	Schank et al. (1981)
			Yes	0	17.3	4.3*	3.7	0.232		1.36	
	Sand		No	0	8.4	2.6	5.8	0.186		2.26	
			Yes	0	10.6*	3.1	5.4	0.196		1.85	
Panicum milaceum	Soil	*A. brasilense* (mixed)	No		0.59	0.48		11.0		1.95	Kapulnik et al. (1981a)
			Yes		0.95*	1.10*		17.9*		1.88	
Sorghum bicolor × *Sorghum sudanense*			No		1.30	0.60		27.4		1.83	
			Yes		1.70*	1.32*		40.5*		2.28	

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
			<--		various units[a]					-->	
Triticum aestivum			No		0.58	0.54					
			Yes		1.09*	1.31*					
Sorghum bicolor			No		1.71	1.21	0	28.0		1.64	
			Yes		2.72**	1.18	61	48.4*		1.78	
Setaria italica	Soil	*A. brasilense* Cd	No	0	0.93			10.69		1.15	Kapulnik et al. (1981b)
			Yes	0	1.76*			18.48*		1.05	
			No	0	0.79						
			Yes	0	0.98*						
			No	0.01	0.70						
			Yes	0.01	0.85*						
			No	0.04	0.83						
			Yes	0.04	1.25*						
			No	0.1	1.01						
			Yes	0.1	1.27*						
			No	0.2	1.08						
			Yes	0.2	1.29*						
Zea mays Sweet September	Soil	*A. brasilense* Sp 7	No	0.01	2.680c		8.8	14.16b		0.530	O'Hara et al. (1981)
			Yes	0.01	3.955a		25.1	16.89abc		0.427	
		JM 6A2	Yes	0.01	3.267b		11.9	13.34c		0.408	
		Sp Br 14	Yes	0.01	3.560ab		53.3	16.05ab		0.445	
		Sp 107st	Yes	0.01	3.480b		3.9	16.14ab		0.462	
		Sp F104	Yes	0.01	3.650ab		0	17.02ab		0.463	
		Sp F105	Yes	0.01	3.822ab		3.5	17.28a		0.452	
		S-631	Yes	0.01	3.910a		46	15.47b		0.395	

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
					various units[a]						
Zea mays			No	0.01	2.619d		2	12.87c		0.491	
John Innes		Sp 7	Yes	0.01	4.052a		25.1	22.30a		0.551	
		JM 6A2	Yes	0.01	2.992c		16.4	17.30b		0.591	
		Sp Br14	Yes	0.01	3.018c		0	15.75b		0.521	
		Sp 107st	Yes	0.01	3.558b		2.5	17.26b		0.485	
		Sp F104	Yes	0.01	3.095c		10.5	15.13b		0.488	
		Sp F105	Yes	0.01	3.692b		0	16.31b		0.441	
		S-631	Yes	0.01	3.304bc		21	17.47b		0.533	
Triticum durum	Soil	*A. brasilense*	No	Low	1.33					1.53	Avivi and Feldman (1982)
Responsive		(mixed)	Yes	Low	2.05**					1.49	
Nonresponsive			No	Low	1.82					1.50	
			Yes	Low	1.97					1.53	
Triticum aestivum			No	Low	1.14						
			Yes	Low	1.5*						
Triticum aestivum	Soil	*A. brasilense*	No	0	1.10	0.73	25.2			0.43	Hegazi (1982)
		+regulators	No	0	0.48	0.65	12.9			0.57	
		EgW2	Yes	0	1.10	0.83	177.0			0.41	
		WgG1	Yes	0	1.20	0.95	307.6			0.41	
		EgW2	Yes	500	1.89	0.65	39.2			1.19	
		WgG1	Yes	500	1.87	0.69	44.2			1.11	
			No	1000	1.73	0.59	16.5			1.77	

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
				<-- various units[a] -->							
Pennisetum americanum	Soil	*A. brasilense*	No	0	4.20	1.62	4.57				Venkateswarlu and Rao (1983)
		S-7	Yes	0	4.93*	2.57*	5.18				
		S-14	Yes	0	5.95*	3.57*	4.17				
		S-51	Yes	0	4.08	1.67	4.62				
		S-54	Yes	0	5.36*	3.35*	5.57				
		S-59	Yes	0	4.60	2.35*	5.97				
	Solution		No	0.5m*M*	577	131	0.97	8.19		1.42	
		S-7	Yes	0.5m*M*	597	135	4.50	8.36		1.40	
		S-14	Yes	0.5m*M*	723*	187*	5.88	10.12*		1.40	
		S-51	Yes	0.5m*M*	633	136	5.95	8.99		1.42	
		S-54	Yes	0.5m*M*	700*	157	5.64	9.73*		1.39	
		S-59	Yes	0.5m*M*	610	137	3.65	8.72		1.43	
Zea mays	Soil	*A. lipoferum* (mixed)	No		11.4		26.3			0.71	Hegazi 5t al. (1983)
			Yes		22.9*		114.2			1.12	
			No	Straw	20.1		102.7			1.06	
			Yes	Straw	39.1*		144.7			1.39	
Zea mays	Soln	*A. brasilense*	No	N limiting	75.7						Lin et al. (1983)
		Cd	Yes	N limiting	95.9*						
		Sp 7	Yes	N limiting	101.7*						

Table 3. *Continued*

Crop	Medium	Bacterium	Inoculation	Fertilizer N	Yield	Roots	N fixation	N uptake	^{15}N uptake	N content (%)	Reference
			<-- various units" -->								
Sorghum			No	N limiting	6.28						
bicolor		Cd	Yes	N limiting	7.28						
		Sp 7	Yes	N limiting	7.57*						
Triticum	Soil	*A. brasilense*	No		712			3.76			Lethbridge and
aestivum		Sp 107	10ex9		640			3.76			Davidson
			10ex10		80[illegible]*			4.95			(1983)
			10ex11		102[illegible]*			14.53			
			10ex11		125[illegible]*			17.24			
Rescue	Soil Arbuthnott 0.25% N		Dead	14	2.39			12.70	5.20		
			Live	14	2.43			13.30	5.46*		
C-R5D			Dead	14	2.32			13.20	5.34		
			Live	14	2.5*			13.40	5.64*		
R-C5D			Dead	14	2.35			13.70	5.61		
			Live	14	2.5*			13.40	5.47*		
Cadet			Dead	7	1.82			12.50	2.29		
			Live	7	1.89			13.10	2.38		
			Dead	14	2.19			13.10	5.18		
			Live	14	2.06*			13.00	5.26		
			Dead	28	2.31			12.70	10.50		
			Live	28	2.25			13.10	11.10*		
			Dead	56	2.67			11.80	22.80		
			Live	56	2.61			12.10	23.20*		
			Dead	112	3.64			10.70	47.90		
			Live	112	3.37*			11.50	47.30		