P. G. Hunt

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Microbial Responses in the Rhizosphere of Agricultural Plants

The interactive relationship between plant roots and microorganisms was recognized and expressed in 1904 by Lorenz Hiltner, soil bacteriologist and professor of agronomy at the Technical College of Munich. In early studies, plant pathogenic and symbiotic processes that produced morphological changes visible to the unaided eye were the most intensively investigated relationships. Today, study of microbial ecology in plant rhizospheres is extremely broad and complex. Plant and microbial relationships are, of course, affected by the total biophysical system of both the plant and the soil. The system might be viewed as progressive development of interactions among soil structure, growing roots, rhizosphere microorganisms, soil and canopy environments, and the physiology of the entire plant. Consequently, the holistic understanding of rhizosphere microbial ecology has become vitally important to soil management and plant growth.

Other chapters in this volume focus on specific aspects such as soil structure, mycorrhizae, plant pathogenicities, nitrogen cycling, soil fauna, and shoot/root development. The emphasis of this chapter will be microbial involvement in soil structure and nitrogen fixation in the rhizospheres of plants grown in agricultural systems. Only a few of many studies will be used to illustrate particular points; thus, some excellent investigations may not be cited.

RHIZOSPHERE ESTABLISHMENT AND SOIL STRUCTURE

Root growth is fast in young seedlings. The pattern, rate, and extent of root development is, of course, dependent upon the genetic makeup of the plant and environmental conditions. For instance, the rooting forces necessary to penetrate soil layers are directly related to the soil strength characteristics, which are dramatically affected by soil structure and water content. Rooting in many soils may occur only when the soil is wet enough to promote low strength characteristics but dry enough to have adequate O_2 contents (Campbell, Reicosky, and Doty 1974; Campbell and Phene 1977). Thus, microbial promotion of soil structure prior to, as well as in conjunction with, root growth is very important.

The production of extracellular polysaccharides is predominantly a bacterial process, and it promotes what can be referred to as the "cementing" of particles. Chaney and Swift (1986) reported that soil aggregates were reformed by polysaccharides produced from glucose amendment but not by wetting/drying and freezing/thawing cycles. These aggregates declined over a twelve-week period as polysaccharides were decomposed. The stability of these aggregates was related to the original organic matter levels in the soils. Chapman and Lynch (1984) found that the polysaccharides of straw were composed mainly of galactose, glucose, and mannose. The ability of straw to increase aggregation was also shown to be inversely related to straw nitrogen contents between 0.25 and 1.09 percent by Elliott and Lynch (1984).

The use of bacteria and fungi in a coculture was described by Chapman and Lynch (1985). They found increased aggregate stability of a Humble silt loam with the coinoculation of _Trichoderma harzianum_ and _Enterobacter cloacae_. They found that the cellulolytic fungi could support the growth of bacteria and supply sufficient carbon for polysaccharide synthesis. Anaerobic and facultative anaerobic nitrogen-fixing bacteria were thought to be sustained by cellulolytic enzymes of aerobic fungi (Harper and Lynch 1984). Fungi play a major role in the stability of soil aggregates as their mycelia grow around soil particles and ridge to others. This bridging is of increased importance once the root grows near the soil particles. Fungi also play a role in the transport of chemicals among microsites (Newman 1985).

Once the root occupies an area of the soil, it affects and is affected by the microorganisms of that region. Thus, the root has an immediate effect on the microbial ecology of the soil (Figure 5.1). Roots excrete large amounts of photosynthate (Lynch et al. 1981), and these organic compounds serve as substrates for microbial populations. The plant roots and shoots are, in turn, affected by the microbes. For example, Barber and Lynch (1977) found that the biomass of bacteria produced in the rhizosphere of barley seedlings was greater than could have been produced from the carbohydrates released in sterilized soil. These data supported the view that microbes do not simply grow on plant roots; they stimulate plants to release more photosynthate. Barber and Martin (1976) estimated that the exudate losses of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) seedlings were equivalent to 7 to 13

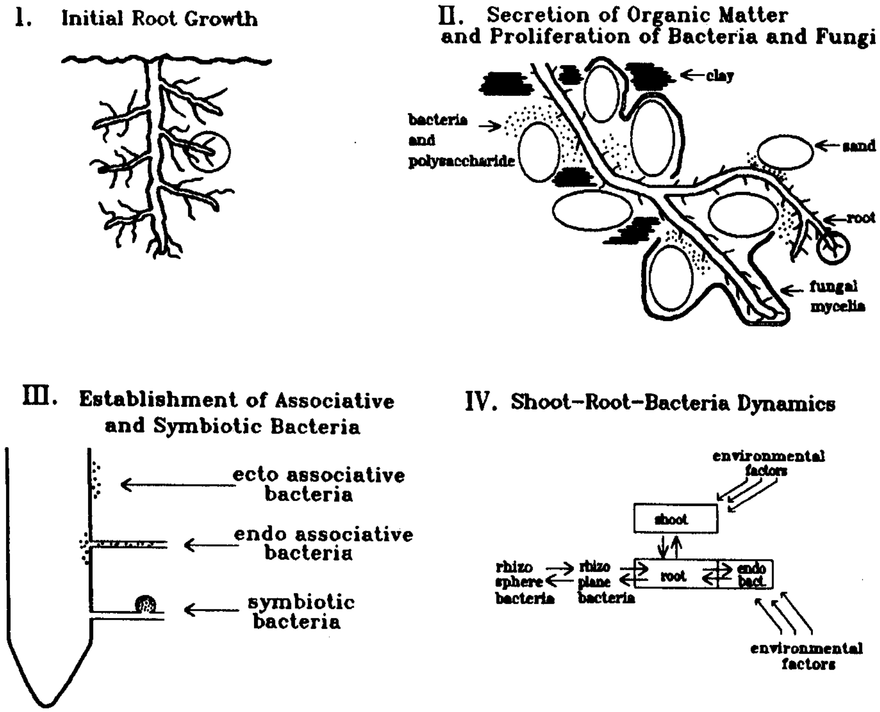

Figure 5.1. A schematic of root development, rhizosphere establishment, and shoot-root-microbial equilibrium.

percent and 18 to 25 percent of their dry matter under sterile and nonsterile conditions, respectively. Microbially produced growth regulators may also stimulate increased total growth of the plant. Beck and Gilmour (1983) found that wheat released 3.7 and 3.0 percent of the ^{14}C-labeled photosynthate as soluble exudates when inoculated and noninoculated with nitrogenase-positive bacteria, respectively. Whipps and Lynch (1983) concluded that competition for available root exudate on the root of barley would be high and that it would preclude luxuriant bacterial growth in the rhizosphere.

The degree of the interrelation between the root and microbe varies. Those microorganisms that live within or in close association with the root are most directly affected by the plant. This region can be called the endorhizosphere. Many microbes also occupy the rhizoplane, which is the actual root surface that contacts the soil, water, and air. The third zone is the exorhizosphere, which is separated from the root by a film of water. A good view of these environments was expressed by Whipps and Lynch (1983). They suggested that regions can best be viewed as a continuum that is by nature dynamic with plant growth.

In addition to microbial effects, physical and chemical factors such as soil texture, structure, color, organic content, water status, pH, and salinity play major roles in the expression of rooting potential. These factors along with variations in plant canopy condition make the rhizosphere quite variable from plant to plant as well as from field to field.

ASSOCIATIVE NITROGEN FIXATION

Associative nitrogen-fixing microorganisms have been a source of interest for many years. Earlier work with Azotobacter inoculation has been controversial (Brown 1972). Several rhizoplane genera such as Bacillus, Enterobacter, Pseudomonas, and Beijerinckia are capable of nitrogen fixation in the rhizosphere of grain crops such as corn (Zea mays), wheat, sorghum (Sorghum bicolor), and rice (Oryza sativa) as well as in the rhizosphere of tropical forages (Neyra and Dobereiner 1977; Hubbell and Gaskins 1984). Dobereiner (1961) was one of the first to galvanize recent thought about the use of associative nitrogen fixation when she showed that Beijerinckia was stimulated by the sugar-cane (Saccharum officinarum) rhizosphere. Subsequently, Dobereiner and Day (1976) reported nitrogen fixation in the rhizosphere of Digitaria decumbes by the bacterium now known as Azospirillum.

A complete discussion of associative nitrogen fixation is far beyond the scope of this chapter. The genus Azospirillum has received a great deal of world-wide attention in recent years. Its two well-studied species, A. brasilense and A. lipoferum, are good generic examples of bacteria that grow in the rhizosphere, fix nitrogen, and produce plant growth regulators.

Occurrence of Azospirillum

In field and greenhouse work, Baldani and Dobereiner (1980) found that A. lipoferum was much more commonly isolated from externally sterilized roots of maize (Zea mays) than from wheat, but the reverse was true for A. brasilense. In Hawaii, Kosslak and Bohlool (1983) found A. brasilense and A. lipoferum to be present in a number of plants in about equal percentages. In their review of associative nitrogen fixation by Azospirillum, Hubbell and Gaskins (1984) suggested that Azospirillum was generally found in associations with plants where investigators looked for them and that the frequent discovery of Azospirillum on the roots of tropical grasses might be more of a quantitative than a qualitative nature.

Plant Responses to Associative Bacteria

O'Hara, Davey, and Lucas (1981) reported that seven strains of A. brasilense increased the dry matter and nitrogen content of maize shoots when they were grown under temperate conditions in sand-filled vermiculite pots. The positive response of plant growth to A. brasilense inoculation in India has been reported by Rai (1985a,b,c). The positive response in seed yields were particularly evident when soil and fertilizer nitrogen were low. Strains of A. brasilense that were adapted to the prevailing soil environments were most effective in increasing corn, cheena (Panicum millaceum), and millet (Eleusine coracana) growth and yield. Also in India, Meshram and Shende (1982) reported increased grain yield as well as increased nitrogen content in maize grain and stover after the inoculation of maize with A. chroococcum. Rai and Gaur (1982) reported that maize yields were 2.97 t/ha with nitrogen treatment of 80 kg/ha and 4.15 t/ha when *A. lipoferum* inoculant was used along with the same fertilizer nitrogen. The particular strain of *A. lipoferum* had a high nitrogen-fixing capacity and no denitrifying tendency.

Smith et al. (1976) reported that several tropical grasses and cereals of North America experienced enhanced growth when inoculated with *Spirillum lipoferum*. The growth and total nitrogen content of wheat, sorghum, sorghum X Sudan grass (*Sorghum bicolor Sudanense*), and Proso millet *Panicum millaceum*) were increased by inoculation with *A. brasilense* in Israel (Kapulnik et al. 1981a). Yield of summer cereal crops and *Setaria italica* were also increased by inoculation with *Azospirillum* (Kapulnik et al. 1981b).

Yields of winter and spring wheat were increased by aerial application of *A. brasilense* in field studies during 1979 and 1980 (Reynders and Vlassak 1982). They believed that the bacteria caused increased tillering and nutrient uptake. Sarig et al. (1984) reported increases in both grain and forage yield for sorghum cultivars grown under dryland conditions in Israel. Smith et al. (1976) reported increases in the herbage produced by Pearl millet (*Pennisetum americanum*) and Guinea grass (*Panicum maximum*) when they were inoculated with *A. brasilense*, but a majority of forty tropical grasses tested did not show increases in growth.

Nitrogen Fixation

The amount of nitrogen obtained from associative fixation by *Azospirillum* spp. has been estimated at different levels, and some of the very high estimates are no doubt due to an overestimation bias of the acetylene reduction method (Gaskins and Carter 1975; van Berkum and Bohlool 1980; von Bulow and Dobereiner 1975). Apparent overestimations have occurred in other associations. Brown (1976), for example, also reported that the main advantage of *Azotobacter paspali* in the rhizosphere of *Paspalum notatum* was the production of growth regulators rather than nitrogen fixation. This is in contrast to estimates of as much as 90 kg/ha nitrogen fixation by *A. paspali* on *P. notatum* by Dobereiner, Day, and Dart (1972).

Production of Growth Regulator Substances

The low nitrogen fixation rates of associative nitrogen-fixing bacteria support the view that much, if not all, of the plant growth stimulation derived from associative nitrogen-fixing bacteria comes from plant growth hormones rather than nitrogen fixation (Tien, Gaskins, and Hubbell 1979; Tien et al. 1981; Lin, Okon, and Hardy 1983). In either case, it appears that associative nitrogen-fixing bacteria can often increase the yield of important forages and grains, especially when soil fertility is low and the strains are adapted to restrictive environmental conditions such as pH, salinity, or temperature.

SYMBIOTIC NITROGEN FIXATION

Biological nitrogen fixation is certainly among the most important rhizosphere processes. Symbiotic nitrogen fixation was a cornerstone of soil fertility until the development of chemical conversion of atmospheric nitrogen to ammonia; chemical conversion gave the world a new source of nitrogen fertilizer which greatly increased agricultural productivity. Nitrogen fertilizers are not, however, panaceas. Conversion of atmospheric N^2 to ammonium is an energy-intensive process, and fertilizers so produced must be transported to locations of agricultural use. This transportation is expensive and energy consumptive at best, and not available in many areas of the world. Additionally, overuse of nitrogen in areas such as the midwestern section of the United States has led to groundwater contamination. Thus, there is continued interest in legume-rhizobial symbiosis.

For an effective legume-rhizobial symbiosis, the rhizobia must be present, infection/nodule formation must occur, and the rhizobia must be an efficient nitrogen fixer within the plant. Rhizosphere conditions impact all of these critical aspects of dinitrogen fixation.

Rhizobial Presence and Survival

Rhizobia that are capable of infecting and forming nodules in a particular legume must be present or introduced into a soil if effective symbiosis is to be established. Cropping and farm management practices will, of course, have dramatic impact on the number of rhizobia present. For example, Weaver, Frederick, and Dumenil (1972) determined that the number of Bradyrhizobium japonicum cells in fifty two Iowa fields was positively correlated to the presence of soybean (Glycine max) in the crop rotation during the previous thirteen years. Hiltbold, Patterson, and Reed (1985) found that the rhizobial numbers of B. japonicum were high (10^6 cell g^{-1}) during the winter following soybean production. If lime, phosphorus, and potassium were adequate, rhizobial numbers stabilized around 10^4 or 10^5 cells g^{-1} of soil. Soil acidity reduced rhizobial populations. When pH levels were less than 4.6, rhizobial numbers were generally less than 10^2 g^{-1} of soil. Rupela et al. (1987) found that research station soils in India contained from 10^3 to 10^5 rhizobia cells g^{-1} of soil, while those of farmers contained from 10 to 10^3 rhizobia g^{-1} of soil. Populations of rhizobia were highest during and shortly after the growth of a legume crop. Populations then decreased to a lower level, but levels were generally quite sufficient for inoculation of subsequent crops unless extreme environmental conditions were present.

Rhizobial populations can be greatly affected by factors such as water content (Pena-Cabriales and Alexander 1979), temperature (Munevar and Wollum 1981; Osa-Afiana and Alexander 1982; Kvien and Ham 1985), pH (Keyser and Munns 1979b), salinity (Rai et al. 1985; Rai 1987), and nutrient status of the soil (Keyser and Munns 1979a,b). As a result, some areas planted to legumes may not be high in numbers of rhizobia or percentage of effective nodulators. For example, cowpea (Vigna unguiculata) rhizobial populations of Guyana soils were low enough in both numbers and effectiveness for potential benefit from inoculation with effective strains (Trotman and Weaver 1986). Rao et al. (1985) reported that rhizobia used to inoculate the American soybean cultivar "Bossier" in Nigerian soils were found in soybean grown after two years of fallow, but the yield of the soybean was increased by annual inoculation. Survival of the rhizobia may even be inhibited by the seed as in the case of R. trifolii and arrowleaf clover (Trifolium vesiculosum) (Materon and Weaver 1984). Fuhrmann, Davey, and Wollum (1986) found differences in the effects of desiccation on R. leguminosarum bv. trifolii on a Altavista loamy sand and a Cecil sandy clay loam. Population levels were generally lowered by incubation at the -70 MPa level, but one isolate had excellent survival under all soil moisture treatments, including the -500 MPa moisture level. Wollum and Cassel (1984) used geostatistical techniques to assess the spatial variability of rhizobial populations. Large population variations occur not only from field to field and soil to soil but over small distances in the same field and soil.

Infection and Nodule Formation

Rhizobia have been classified by their ability to inoculate certain plants, but this has not been entirely satisfactory. Certain rhizobia are capable of infecting and nodulating plants of several species, and certain plants are nonselective in their nodulation. In addition, rhizobia have been classified by their rate of growth on synthetic media, i.e., fast- or slow-growing (Jordan 1982; Sadowsky, Keyser, and Bohlool 1983). It is also important to understand that infection involves both the plant and the microorganism. Even after recognition and infection by the rhizobia, plants can regulate subsequent nodulation by the same or other rhizobia (Kosslak and Bohlool 1983), and this regulation can be affected by environmental conditions as subtle as the spectral composition of canopy light (Kasperbauer, Hunt, and Sojka 1984; Hunt, Kasperbauer, and Matheny 1987).

One of the most difficult problems associated with improvements in the legume/rhizobia symbiosis in agricultural systems is the low competitiveness of introduced strains of rhizobia relative to indigenous strains. This is true even if the introduced strain will readily infect and form nodules on a plant when the strain is present in a single culture. Differences in infection potential can be seen from the fact that serogroup 123 dominated soybean nodules with 60 to 100 percent occupancy, even though it did not dominate the rhizobial population of the rhizosphere; the numbers of B. japonicum serogroups 110, 123, and 138 all increased in the rhizosphere of soybean to about 10^6 cells g^{-1} soil (Moawad, Ellis, and Schmidt 1984). Populations of serogroups 110, 123, and 138 in fallow soil were each about 10^5 cells g^{-1} soil. Moawad and Bohlool (1984) found that strain B214 was least competitive among six strains for nodulation of Leucaena leucocephala in an oxisol (less than 30 percent) but most competitive in a mollisol (70 percent). The correlation between occupation of nodules and rhizosphere populations was low. Competitive advantages of various rhizobia not only change with soils, but with tillage and cultivar on the same soil (Hunt, Matheny, and Wollum 1985). They concluded that tillage, cultivar, and inoculation interacted to influence *B. japonicum* strain occupancy (Table 5.1).

Weaver and Frederick (1974) found that introduced strains must be present in numbers 10^3 or 10^4 greater than the indigenous strains to significantly impact nodule occupancy of soybean. Even these high numbers often produced less than 10 percent nodule occupancy in field inoculation studies in the southeastern United States (Hunt et al. 1983). Dunigan et al. (1984) initially found that infection of soybean by *B. japonicum* strain USDA 110 was low (0 to 17 percent) even when massive inoculations (10^8 cells cm^{-1}) of *B. japonicum* were used for three consecutive years. During the subsequent four years, however, the percentage of nodules infected by the introduced strain increased to as high as 60 percent; the researchers interpreted this to indicate that a prolonged application of massive inoculation would result in the establishment of the introduced strain as a significant portion of the rhizobial population. It is possible that prolonged use of quality inoculum may become an important practice, but substantial progress must be made in the uniformity and quality of inoculum (Giddens, Dunigan, and Weaver 1982).

Viteri and Schmidt (1987) reported that indigenous soil rhizobia could respond to the addition of several sugars, particularly arabinose; *B. japonicum* populations increased from about 10^4 to about 10^6. They interpreted this as evidence that indigenous populations of rhizobia could respond to various substrates in the absence as well as in the presence of a host plant.

Trinick, Rhodes, and Galbraith (1983) found that the fast- and slow-growing rhizobia competed differently under temperatures of 25 and 30° C. They also reported a difference in competitiveness with variation in the day/night temperatures. This is of considerable importance because it represents the normal fluctuation of the daily temperature cycle and the differences that exist with different soil and water management systems, i.e. crop residue management, water management, and tillage. Rai and Gaur (1982) and Rai (1987) reported selection of lentil rhizobia that had improved temperature, pH, and salinity tolerance. Mahler and Wollum (1981) reported differences in the drought tolerance of *B. japonicum* strain in the symbiotic as well as in the free-living state.

Differences also are found among strains for phage tolerance. A rhizobiophage of *B. japonicum* USDA 117 was found to reduce the nodule number, nodule weight, and acetylene-reduction capacity of plants inoculated with USDA 110

TABLE 5.1. Nodular occupancy by eight *Bradyrhizobium japonicum* strains as affected by soybean cultivar, tillage, and inoculation with strain 110 of *B. japonicum*.

Tillage	Inoculation	*Bradyrhizobium japonicum* strains							
		24	31	46	76	94	110	122	125
		%							
1980									
					Lee				
Conventional	+	3	7	4	3	3	7	8	2
	-	2	11	1	8	5a	4	8	0
Conservation	+	1	6	1	8	10b	5	3	6a
	-	1	13	5	7	0ab	0	6	1a
					Ransom				
Conventional	+	1	22	4	7	0	12	6	0
	-	0	16	2a	7	0	9	4	0
Conservation	+	3	21	4	6	0	7	5b	0
	-	0	28	9a	4	0	3	0b	0
					Coker				
Conventional	+	1	10	8	12	9	3	4	2
	-	0	19	6	8	14	4	4	0
Conservation	+	0	13	3	12	11	2	11	1
	-	0	16	9	7	10	2	4	1
CVd		77	35	39	31	44	56	48	59

1981									
					Lee				
Conventional	+	9	8	6a	1b	9b	2	13	0
	-	13	6	5	4b	20b	0	8	0
Conservation	+	11	4	0ab	0c	20	4b	6	0
	-	20	2	4b	4c	10	0b	7	0
					Ransom				
Conventional	+	7	14b	6	1ab	14	2	3ab	0
	-	5	5b	7	5b	26	0	11b	0
Conservation	+	9	14	6	6a	11	0b	8a	0
	-	12	8	6	7	12	3b	9	0
					Coker				
Conventional	+	13a	7a	6b	3	12	1a	11	1
	-	15	6a	1b	2	18	0	6	0
Conservation	+	15	6a	7c	8b	26	6ab	10	0
	-	10b	15ab	1c	3b	16	2b	8	0
CV[d]		36	30	41	44	31	60	28	29

[a,b,c] Means for the same year, serogroup, and cultivar followed by "a" are different for tillage and those means followed by "b" or "c" are different for inoculation by the LSD test at the 0.10 level when analyzed after a square root of (mean + 0.5) transformation.

[d] CV values were calculated from transformed data; therefore, means within a column should be transformed by a square root of (mean + 0.5) before comparing to CV values.

Source: Hunt, Matheny, and Wollum 1985.

(Hashem and Angle 1988).

Iron deficiency was found to inhibit the nodule development of ground nut (Anachis hypogaea) in calcareous soil of Thailand (O'Hara et al. 1988). However, iron deficiency did not limit growth or populations of ground nut Bradyrhizobia in the soil or rhizosphere. Whelan and Alexander (1986) found that R. trifolii did not nodulate subterranean clover (Trifolium subterraneum) in the presence of high levels of iron or aluminum (500 and 50 micromoles, respectively) nor below pH 4.8. Riley and Dilworth (1985) reported that the adverse effect of cobalt deficiency on nitrogen fixation in Lupinus angustifolius was due to the inability of the plant to supply cobalt to the rhizobia rather than an effect on their growth in soil.

Many postulates have been made about why introduced strains are less competitive than indigenous strains. Greater environmental tolerance, more homogenous distribution in the soil, and improved recognition abilities are possible reasons. Selection of plants that will exclude predominant rhizobial strains has been done (Cregan and Keyser 1986), but there are many questions that must be answered before such a process can be used agronomically. At least partial resolution of the problem could rest with improved planting environments and inoculants, i.e., favorable soil water status, neutral pH, low salinity, adequate nutrition, and moderate temperature, along with large numbers of viable cells in the inoculum.

Efficient Nitrogen Fixation

Young, Hughes, and Mytton (1986) found dramatic increases in dry matter production during the first year after inoculation of white clover (Trifolium repens) with R. trifolii; and such results are not uncommon. Yet, strains of rhizobia that carry out efficient nitrogen fixation in one plant may fail to do so in another. In some plants, infection and nodulation occur, but nitrogen fixation is nonexistent or ineffective (Mathis, Kuykendall, and Elkan 1986). Under these conditions, the rhizobia acts as a parasite. It is using photosynthate to grow, but it is providing no nitrogen for plant growth. Keyser et al. (1982) reported a very interesting ineffective infection of fast-growing rhizobia (generation times of two to four hours) with soybean. They isolated these rhizobia from wild progenitor soybean (Glycine soja) in China. Soybean (Glycine max) is normally only infected by slow-growing rhizobia (generation times of more than six hours). The nodules formed by the fast-growing rhizobia were only effective in the cultivar "Peking," a black-seeded, genetically unimproved line from China.

There also exists distinct differences in the nitrogen fixing capacities of legume species. Differences between a forage legume such as alfalfa (Medicago sativa) and the common bean (Phaseolus vulgaris) are generally perceived. There also exist more subtle differences among grain legumes; Piha and Munns (1987a,b) found that common beans obtain relatively less nitrogen from fixation than soybean or cowpea. The same was true for several Phaseolus spp., and Piha and Munns concluded that common bean species may be genetically predisposed to lower nitrogen fixation.

The contribution of legumes to the annual nitrogen balance of a soil depends upon several factors, such as the crop, soil type, rhizobial effectiveness, rainfall pattern, residue management, seed yield, and seed nitrogen content. If a crop such as alfalfa is plowed under rather than harvested, the addition of nitrogen to the soil can be several hundred kg/ha (Heichel 1987). If it is harvested several times, however, it may in fact be a net consumer of soil nitrogen. Soybean grown in the midwestern United States is generally a net annual consumer of nitrogen, while that grown in the southeastern United States is generally a net nitrogen producer (Welch et al. 1973; Hunt, Matheny, and Wollum 1985; Thurlow and Hiltbold 1985). This is predominantly the result of soil-nitrogen differences; naturally low levels of soil nitrogen in the southeastern United States allow the fixation and accumulation of large amounts of nitrogen in legumes.

Plant Growth and Seed Yield

If nitrogen is the limiting plant growth and seed yield factor, establishment of a more effective nitrogen-fixing symbiosis will definitely improve seed yield. This has been shown by the marked increase in dry matter and seed yield of nodulating relative to nonnodulating soybean grown in the nitrogen-limiting soils of the southeastern United States (Matheny and Hunt 1983; Thurlow and Hiltbold 1985; Hunt, Matheny, and Wollum 1985). The amount of nitrogen obtained from fixation, however, can be increased without an increase in seed yield (Morris and Weaver 1983; Williams and Phillips 1983), and yield can be increased from rhizobial inoculation without increased nitrogen fixation (Karlen and Hunt 1985; Hunt, Matheny, and Wollum 1985; Hunt et al. 1985). This is possible because the rhizobia are in an interactive state with the plants, other microorganisms, and the environment as depicted in phase IV of Figure 5.1. Under these conditions, the rhizobia are affecting the reaction of plants to the environment by means other than nitrogen fixation, most likely via growth-regulating hormones. This may be more evident in rhizobia with the capacity to produce higher levels of hormones such as indole-3-acetic acid (IAA), but initial field studies with such rhizobia indicate that they do not greatly alter the growth of soybean under field conditions (Kaneshiro and Kwolek 1985; Hunt, Kaneshiro, and Matheny 1987). However, the addition of a low concentration of precursor to the rhizosphere of plants inoculated with growth regulator-producing microorganisms, may cause substantial growth alterations (Frankenberger and Poth 1987). It is even possible that rhizobia may stimulate the germination of nonlegumenous plants such as wheat or corn (Kavimandan 1986).

Interactions of B. japonicum strain with irrigation have been reported (Hunt, Wollum, and Matheny 1981, Hunt et al. 1983, and Hunt, Matheny, and Wollum 1988 (Table 5.2). The soybean seed yield response to irrigation varied with cultivar and strain. They also found differences in seed yield of soybean grown under drought conditions when the soybean was inoculated with cultures of the B. japonicum strain USDA 110 that had been maintained in different laboratories. The effects of canopy configuration (row width and compass orientation) of soybean were accentuated or diminished by the strain of B. japonicum (Hunt et al. 1985, 1990) (Figure 5.2).

Row orientation and strain of rhizobium were also important to the nitrogen fixation and seed yield of chickpea (Cicer arietinum) in India (R. Rai, PL-480 report and personal discussions 1988). The effects of row orientation and soil color on soybean nodulation were postulated to be related to the spectral composition of canopy light, which affected shoot:root ratios, the extent of nodulation, and the relative competitiveness of various strains (Kasperbauer and Hunt 1987, 1988; Kasperbauer, Hunt, and Sojka 1984; Hunt, Kasperbauer, and Matheny 1987, 1989) (Table 5.3). The autoregulation of nodulation expressed by one side of a split root system on the other could be increased or decreased by Red (R) or Far-red (FR) end-of-day light treatment. Since FR effect could be reversed by R, the autoregulation was partially controlled by the plants phytochrome system. Additionally, when soybean were inoculated with B. japonicum strains USDA 110 or Brazil 587 and treated with (R) or (FR) light nodulation was significantly altered if the inoculant was USDA 110 but not if the inoculant was Brazil 587 (Hunt et al. 1990). Thus, the light quality environment of the shoot is able to effect the relative competitiveness of rhizobia in the rhizosphere. It is possible that some of the more important advances in legume seed yield may come from a better understanding of the whole plant response to the rhizobia rather than simple improvements in nitrogen fixation efficiency.

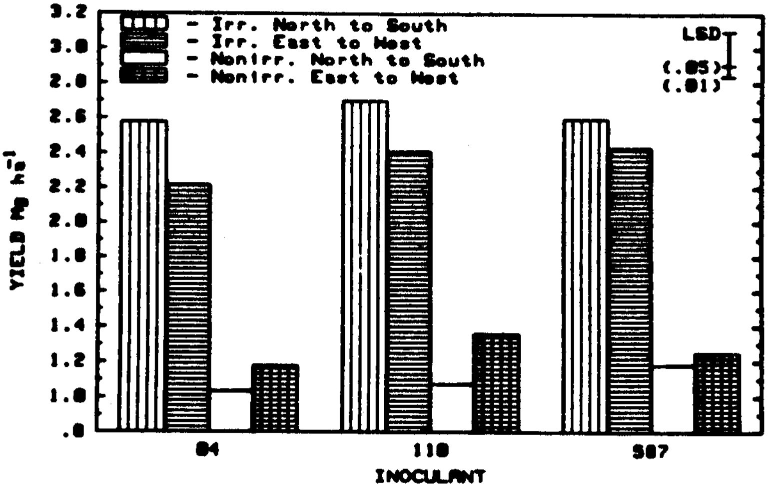

Figure 5.2. Coker 338 soybean yield response to row orientation, inoculation, and irrigation in 1982.

SUMMARY

In summary, it has been the author's intent in this chapter to show the close, continual, and dynamic relationships that exist among plants, rhizosphere microorganisms, and the environment, with emphasis on rhizosphere establishment and nitrogen fixation processes. These interrelations are somewhat cyclic and may be thought of as starting when soil microorganisms begin to degrade crop residues, produce polysaccharides, and build soil structure. Soil structure is important to the rate and extent of root development in subsequent crops, and the interaction of rhizosphere bacteria and fungi are critical

TABLE 5.2. Two-year mean seed yield and seed yield rank for soybean inoculated with different *B. japonicum* strain and grown under irrigated (+) and nonirrigated (-) conditions in 1981 and 1983.

		Bradyrhizobium japonicum strains									
Cultivar	Irr.	1001	1004	1005	1010	1029	110	587	Nat	T102	T184
		-----------------------Mg ha-1	-----------------------------								
Cobb	-	1.08	1.50	.89	1.17	1.28	.98	.85	.93	.83	1.03
	+	1.68	1.40	1.74	1.97	1.96	1.50	1.72	1.75	2.01	1.80
Coker 448	-	1.19	1.47	1.89	1.97	1.75	1.60	1.46	1.17	1.12	1.28
	+	2.32	2.94	2.38	3.03	2.07	2.92	2.53	2.59	2.52	2.37
LSD(0.05)=0.59											
LSD(0.01)=0.77											
		-----------------------Rank	-----------------------------								
Cobb	-	101	143	78	110	111	92	78	85	77	97
	+	146	129	162	179	178	137	153	156	172	165
Coker 448	-	109	132	163	171	158	139	122	98	92	113
	+	186	258	204	270	184	253	235	225	212	192
LSD(0.05)=57											
LSD(0.01)=75											

Source: Hunt, Matheny, and Wollum 1988.

TABLE 5.3. Effects of red (R) and (far-red) FR light treatment of shoots and root inoculation time on nodule number for soybean grown in a split-root system.

Inoculation schedule[b]	Light treatment					
	R		FR		FR,R	
	Side		Side		Side	
	A	B	A	B	A	B
	No./plant*					
A_0B_0	17±4[a]	17±3[a]	8±2[a]	11±1[a]	11±4[a]	12±6[a]
$A_{6-9}B_{6-9}$	27±6[a]	22±4[a]	21±5[a]	19±4[a]	24±5[a]	18±5[a]
A_0B_{2-4}	32±5[a]	14±3[a]	14±3[a]	5±2[a]	18±4[a]	11±3[a]
A_0B_{4-6}	38±5[a]	7±2[a]	16±2[a]	2±1	29±8[a]	4±3[a]
A_0B_{6-9}	38±7[a]	2±2	21±4[a]	0±0	32±8[a]	0±0
A_0B_9	39±8[a]	0±0	22±5[a]	0±0	26±5[a]	0±0
Noninoc	0±0	0±0	0±0	0±0	0±0	0±0

*Values are means ± SE.

[a] Numbers within the same column are significantly different from the noninoculated control at P compared by a single degree of freedom contrast. Numbers were transformed by Box-Cox transformation = 0.11 for homogeneity of variance before analysis.

[b] Letters indicate side of the split root and numbers indicate inoculation times in days after time zero.

Source: Hunt, Kasperbauer, and Matheny 1987.

aspects of enhanced soil structure during the crop growth periods. Once the root grows into the soil microsites, rhizosphere bacteria stimulate production of root exudate, which in turn is used by the microbes to produce more growth-stimulating materials and polysaccharides that can cement and stabilize soil particles. Fungi continue the process by bridging soil aggregates and roots. Associative and symbiotic bacteria become established and promote plant growth by growth regulator production as well as nitrogen fixation. At this point, interactions of the plant, rhizosphere microorganism, and environment of both the shoot and root are dynamic; changes in one part of the system affect all parts of the system. For instance, plant responses to changes in factors such as water status and spectral composition of canopy light are interactive with the presence and competitiveness of rhizosphere rhizobia.

This dynamic state of continual interaction and adjustment is representative of how microorganisms function and respond in the rhizosphere of agricultural crops. Much progress has been made in the nearly 90 years since Professor Hiltner first discussed these interactive relationships. Yet, we have only begun to understand and apply rhizosphere concepts to modern agriculture. It is indeed possible that discovery and application of new information in the microbial aspects of the rhizosphere will be vital building blocks for profitable and environmentally sustainable agriculture enterprises of the future.

REFERENCES

Baldani, V. L. D., and J. Dobereiner. 1980. Host-plant specificity in the infection of cereals with Azospirillum spp. Soil Biology and Biochemistry 12:433-439.

Barber, D. A., and J. M. Lynch. 1977. Microbial growth in the rhizosphere. Soil Biology and Biochemistry 9:305-308.

Barber, D. A., and J. K. Martin. 1976. The release of organic substances by cereal roots into soil. New Phytology 76:69-80.

Beck, S. M., and C. M. Gilmour. 1983. Role of wheat exudates in associative nitrogen fixation. Soil Biology and Biochemistry 15:33-38.

Brown, M. E. 1972. Plant growth substances produced by micro-organisms of soil and rhizosphere. Journal of Applied Bacteriology 35:443-451.

Brown, M. E. 1976. Role of Azotobacter paspali in association with Paspalum notatum. Journal of Applied Bacteriology 40:341-348.

Campbell, R. B., and C. J. Phene. 1977. Tillage, matric potential, oxygen, and millet yield relations in layered soils. Transactions of the American Society of Agricultural Engineers 20:271-275.

Campbell, R. B., D. C. Reicosky, and C. W. Doty. 1974. Physical properties and tillage of Paleudults in the Southeastern Coastal Plains. Journal of Soil and Water Conservation 29:220-224.

Chaney, K., and R. S. Swift. 1986. The influence of organic matter on aggregate stability in some British soils. Journal of Soil Science 35:223-230.

Chapman, S. J., and J. M. Lynch. 1984. A note on the formation of microbial polysaccharide from wheat straw decomposed in the absence of soil. Journal of Applied Bacteriology 56:337-342.

Chapman, S. J., and J. M. Lynch. 1985. Polysaccharide synthesis by capsular microorganisms in coculture with cellulolytic fungi on straw and stabilization of soil aggregates. Biology and Fertilization of Soils 1:161-166.

Cregan, P. B., and H. H. Keyser. 1986. Host restriction of nodulation by Bradyrhizobium japonicum strain USDA 123 in soybean. Crop Science 26:911-916.

Dobereiner, J. 1961. Nitrogen-fixing bacteria of the genus Beijerinckia derx in the rhizosphere of sugar cane. Plant and Soil 3:211-217

Dobereiner, J., and J. M. Day. 1976. Associative symbiosis and free-living systems. In Proceedings of the first international symposium on N_2 fixation, ed. W. E. Newton and C. J. Nyman, 518-538. Pullman: Washington State University Press.

Dobereiner, J., J. M. Day, and P. J. Dart. 1972. Nitrogenase activity and oxygen sensitivity of the Paspalum notatum-Azotobacter paspali association. Journal of General Microbiology 71:103-116.

Dunigan, E. P., P. K. Bollich, R. L. Hutchinson, P. M. Hicks, F. C. Zaunbrecher, S. G. Scott, and R. P. Mowers. 1984. Introduction and survival of an inoculant strain of Rhizobium japonicum in soil. Agronomy Journal 76:463-466.

Elliott, L. F., and J. M. Lynch. 1984. The effect of available carbon and nitrogen in straw on soil and ash aggregation and acetic acid production. Plant and Soil 78:335-343.

Frankenberger, W. T., Jr., and M. Poth. 1987. Biosynthesis of indole-3-acetic acid by the pine ecotomycorrhizal fungus Pisolithus tinctorius. Applied and Environmental Microbiology 53:2908-2913.

Fuhrmann, J., C. B. Davey, and A. G. Wollum II. 1986. Desiccation tolerance of clover rhizobia in sterile soils. Soil Science Society of America Journal 50:639- 644.

Gaskins, M. H., and J. C. Carter. 1975. Nitrogenase activity: A review and evaluation of assay methods. Soil Crop Science Society of Florida 35:10-16.

Giddens, J. E., E. P. Dunigan, and R. W. Weaver. 1982. Legume inoculation in the Southeastern U.S.A. University of Georgia, Southern Cooperative Series Bulletin no. 283. pp. 35.

Harper, S. H. T., and J. M. Lynch. 1984. Nitrogen fixation by cellulolytic communities at aerobic-anaerobic interfaces in straw. Journal of Applied Bacteriology 57:131-137.

Hashem, F. M., and J. S. Angle. 1988. Rhizobiophage effects on Bradyrhizobium japonicum, nodulation and soybean growth. Soil Biology and Biochemistry 20:69-73.

Heichel, G. H. 1987. Legumes as a source of nitrogen in conservation tillage systems. In Proceedings of the national conference, the role of legumes in conservation tillage systems ed. J. F. Power. Soil Conservation Society of America, University of Georgia, Athens, April 27-29.

Hiltbold, A. E., R. M. Patterson, and R. B. Reed. 1985. Soil populations of Rhizobium japonicum in a cotton corn-soybean rotation. Soil Science Society of America Journal 49:343-348.

Hubbell, D. H., and M. H. Gaskins. 1984. Associative N_2 fixation with Azospirillum. In Biological nitrogen fixation. ed. Martin Alexander, 201-224. New York: Plenum Publishing Corporation.

Hunt, P. G., T. Kaneshiro, and T. A. Matheny. 1987. Influence of IAA-producing B. japonicum on growth and yield of soybean. Agronomy Abstracts p. 185.

Hunt, P. G., M. J. Kasperbauer, and T. A. Matheny. 1987. Nodule development in a split-root system in response to red and far-red light treatment of soybean shoots. Crop Science 27:973-976.

Hunt, P. G., M. J. Kasperbauer, and T. A. Matheny. 1989. Soybean seedling growth responses to light reflected from different colored soil surfaces. Crop Science 29:130-133.

Hunt, P. G., M. J. Kasperbauer, and T. A. Matheny. 1990. Influence of Bradyrhizobium japonicum strain on nodulation of soybean with varying far-red/red ratios in canopy light. Crop Science (In press).

Hunt, P. G., T. A. Matheny, and A. G. Wollum II. 1985. Rhizobium japonicum nodular occupancy, nitrogen accumulation, and yield for determinate soybean under conservation and conventional tillage. Agronomy Journal 77:579-584.

Hunt, P. G., T. A. Matheny, and A. G. Wollum II. 1988. Yield and N accumulation response of late-season determinate soybean to irrigation and inoculation with various strains of Bradyrhizobium japonicum. Communication in Soil Science and Plant Analysis 19:1601-1612.

Hunt, P. G., T. A. Matheny, A. G. Wollum II, D. C. Reicosky, R. E. Sojka, and R. B. Campbell. 1983. Effect of irrigation and Rhizobium japonicum strain 110 upon yield and nitrogen accumulation and distribution in determinate soybeans. Communication in Soil Science and Plant Analysis 14:223-238.

Hunt, P. G., R. E. Sojka, T. A. Matheny, and A. G. Wollum II. 1985. Soybean response to R. japonicum strain, row orientation and irrigation. Agronomy Journal 77:720-725.

Hunt, P. G., A. G. Wollum II, and T. A. Matheny. 1981. Effects of soil water on R. japonicum infection, nitrogen accumulation and yield in Bragg soybeans. Agronomy Journal 73:501-505.

Jordan, D. C. 1982. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing root nodule bacteria from leguminous plants. *International Journal of Systemic Bacteriology* 32:136-139.

Kaneshiro, T., and W. F. Kwolek. 1985. Stimulated nodulation of soybeans by *Rhizobium japonicum* mutant (B-14075) that catabolizes the conversion of tryptophan to indole-3-acetic acid. *Plant Science* 42:141-146.

Kapulnik, Y., J. Kigel, Y. Okon, I. Nur, and Y. Henis. 1981a. Effect of *Azospirillum* inoculation on some growth parameters and N-content of wheat, sorghum and panicum. *Plant and Soil* 61:65-70.

Kapulnik, Y., Y. Okon, J. Kigel, I. Nur, and Y. Henis. 1981b. Effects of temperature, nitrogen fertilization, and plant age on nitrogen fixation by *Setaria italica* inoculated with *Azospirillum brasilense* (strain cd). *Plant Physiology* 68:340-343.

Karlen, D. L., and P. G. Hunt. 1985. Copper, nitrogen, and *Rhizobium japonicum* relationships in determinate soybean. *Journal of Plant Nutrition* 8:395-404.

Kasperbauer, M. J., and P. G. Hunt. 1987. Soil color and surface residue effects on seedling light environment. *Plant and Soil* 97:295-298.

Kasperbauer, M. J., and P. G. Hunt. 1988. Biological and photometric measurement of light transmission through soils of various colors. *Botanical Gazette* 149:361-364.

Kasperbauer, M. J., P. G. Hunt, and R. E. Sojka. 1984. Photosynthate partitioning and nodule formation in soybean plants that received red or far-red light at the end of the photosynthetic period. *Physiologia Plantarum* 61:549-554.

Kavimandan, S. K. 1986. Influence of rhizobial inoculation on yield of wheat (*Triticum aestivum* L.). *Plant and Soil* 95:297-300.

Keyser, H. H., and D. N. Munns. 1979a. Effects of calcium, manganese and aluminum on growth of rhizobia in acid media. *Soil Science Society of America Journal* 43:500- 503.

Keyser, H. H., and D. N. Munns. 1979b. Tolerance of rhizobia to acidity, aluminum, and phosphate. *Soil Science Society of America Journal* 43:519-523.

Keyser, H. H., B. B. Bohlool, T. S. Hu, and D. F. Weber. 1982. Fast-growing rhizobia isolated from root nodules of soybean. *Science* 215:1631-1632.

Kosslak, R. M., and B. B. Bohlool. 1983. Prevalence of Azospirillum spp. in the rhizosphere of tropical plants. Canadian Journal of Microbiology 29:649-652.

Kvien, C. S., and G. E. Ham. 1985. Effect of soil temperature and inoculum rate on the recovery of three introduced strains of Rhizobium japonicum. Agronomy Journal 77:484-489.

Lin, W., Y. Okon, and R. W. F. Hardy. 1983. Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with Azospirillum brasilense. Applied and Environmental Microbiology 45:1775-1779.

Lynch, J. M., J. H. Slater, J. A. Bennett, and S. H. T. Harper. 1981. Cellulase activities of some aerobic micro-organisms isolated from soil. Journal of General Microbiology 127:231-236.

Mahler, R. L., and A. G. Wollum II. 1981. The influence of soil water potential and soil texture on the survival of Rhizobium japonicum and Rhizobium leguminosarum isolates in the soil. Soil Science Society of America Journal 45:761-766.

Materon, L. A., and R. W. Weaver. 1984. Survival of Rhizobium trifolii on toxic and non-toxic arrowleaf clover seeds. Soil Biology and Biochemistry 16:533- 535.

Matheny, T. A., and P. G. Hunt. 1983. Effects of irrigation on accumulation of soil and symbiotically fixed N by soybean grown on a Norfolk loamy sand. Agronomy Journal 75:719-722.

Mathis, J. N., L. D. Kuykendall, and G. H. Elkan. 1986. Restriction endonuclease and nif homology patterns of Bradyrhizobium japonicum USDA 110 derivatives with and without nitrogen fixation competence. Applied and Environmental Microbiology 51:477-480.

Meshram, S. U., and S. T. Shende. 1982. Total nitrogen uptake by maize with Azotobacter inoculation. Plant and Soil 69:275-279.

Moawad, H. A., and B. B. Bohlool. 1984. Competition among Rhizobium spp. for nodulation of Leucaena leucocephala in two tropical soils. Applied and Environmental Microbiology 48:5-9.

Moawad, H. A., W. R. Ellis, and E. L. Schmidt. 1984. Rhizosphere response as a factor in competition among three serogroups of indigenous Rhizobium japonicum for nodulation of field-grown soybeans. Applied and Environmental Microbiology 47:607-612.

Morris, D. R., and R. W. Weaver. 1983. Mobilization of ^{15}N from soybean leaves as influenced by rhizobial strains. Crop Science 23:1111-1114.

Munevar, F., and A. G. Wollum II. 1981. Effect of high root temperature and *Rhizobium* strain on nodulation, nitrogen fixation, and growth of soybeans. *Soil Science Society of America Journal* 45:1113-1120.

Newman, E. I. 1985. The rhizosphere: Carbon sources and microbial populations. *Special Publication Series of the British Ecological Society* 4:107-121.

Neyra, C. A., and J. Dobereiner. 1977. Nitrogen fixation in grasses. *Advances in Agronomy* 29:1-51.

O'Hara, G. W., M. R. Davey, and J. A. Lucas. 1981. Effect of inoculation of *Zea mays* with *Azospirillum brasilense* strains under temperate conditions. *Canadian Journal of Microbiology* 27:871-877.

O'Hara, G. W., M. J. Dilworth, N. Boonkerd, and P. Parkpian. 1988. Iron-deficiency specifically limits nodule development in peanut inoculated with *Bradyrhizobium* sp. *New Phytology* 108:51-57.

Osa-Afiana, L. O., and M. Alexander. 1982. Differences among cowpea rhizobia in tolerance to high temperature and desiccation in soil. *Applied and Environmental Microbiology* 43:435-439.

Pena-Cabriales, J. J., and M. Alexander. 1979. Survival of *Rhizobium* in soils undergoing drying. *Soil Science Society of America Journal* 43:962-966.

Piha, M. I., and D. N. Munns. 1987a. Nitrogen fixation capacity of field-grown bean compared to other grain legumes. *Agronomy Journal* 79:690-696.

Piha, M. I., and D. N. Munns. 1987b. Nitrogen fixation potential of beans (*Phaseolus vulgaris* L.) compared with other grain legumes under controlled conditions. *Plant and Soil* 98:169-182.

Rai, R. 1985a. Manganese-resistance mutants of *Azospirillum brasilense*: Their growth and relative efficiency in associative nitrogen fixation with cheena (*Panicum miliaceum*) in acid soil. *Journal of General Applied Microbiology* 31:211-219.

Rai, R. 1985b. Studies on associative nitrogen fixation by antibiotic-resistant mutants of *Azospirillum brasilense* and their interaction with lentil (*Lens culinaris*) rhizobium strains in calcareous soil. *Journal of Agricultural Science* 104:207-215.

Rai, R. 1985c. Studies on nitrogen fixation by antibiotic resistant mutants of *Azospirillum brasilense* and their interaction with cheena (*Panicum miliaceum*) genotypes in calcareous soil. *Journal of Agricultural Science* 105:261-270.

Rai, R. 1987. Chemotaxis of salt-tolerant and sensitive *Rhizobium* strains to root exudates of lentil (*Lens culinaris* L.) genotypes and symbiotic N-fixation, proline content and grain yield in saline calcareous soil. *Journal of Agricultural Science* 108:25-37.

Rai, R, S. K. T. Nasar, S. J. Singh, and V. Prasad. 1985. Interactions between *Rhizobium* strains and lentil (*Lens culinaris* Linn.) genotypes under salt stress. *Journal of Agricultural Science* 104:199-205.

Rai, S. N., and A. C. Gaur. 1982. Nitrogen fixation by *Azospirillum* spp. and effect of *Azospirillum lipoferum* on the yield and N-uptake of wheat crop. *Plant and Soil* 69:233-237.

Rao, V. R., A. Ayanaba, A.R.J. Eaglesham, and G. Thottappilly. 1985. Effects of *Rhizobium* inoculation on field-grown soybeans in Western Nigeria and assessment of inoculum persistence during a two-year fallow. *Tropical Agriculture* 62:125-130.

Reynders, L., and K. Vlassak. 1982. Use of *Azospirillum brasilense* as biofertilizer in intensive wheat cropping. *Plant and Soil* 66:217-233.

Riley, I. T., and M. J. Dilworth. 1985. Cobalt status and its effects on soil populations of *Rhizobium lupini*, rhizosphere colonization and nodule initiation. *Soil Biology and Biochemistry* 17:81-85.

Rupela, O. P., B. Toomsan, S. Mittal, P. J. Dart, and J. A. Thompson. 1987. Chickpea *Rhizobium* populations: Survey of influence of season, soil depth and cropping pattern. 1987. *Soil Biology and Biochemistry* 19:247-252.

Sadowsky, M. J., H. H. Keyser, and B. B. Bohlool. 1983. Biochemical characterization of fast- and slow-growing rhizobia that nodulate soybeans. *International Journal of Systematic Bacteriology* 33(4):716-722.

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

Smith, R. L., J. H. Bouton, S. C. Schank, K. H. Quesenberry, M. E. Tyler, J. R. Milam, M. H. Gaskins, and R. C. Littell. 1976. Nitrogen fixation in grasses inoculated with *Spirillum lipoferum*. *Science* 193:1003-1005.

Thurlow, D. L., and A. E. Hiltbold. 1985. Dinitrogen fixation by soybeans in Alabama. *Agronomy Journal* 77:432-436.

Tien, T. M., H. G. Diem, M. H. Gaskins, and D. H. Hubbell. 1981. Polygalacturonic acid transeliminase production by *Azospirillum* species. *Canadian Journal of Microbiology* 27:426-431.

Tien, T. M., M. H. Gaskins, and D. H. Hubbell. 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.

Trinick, M. J., R. L. Rhodes, and J. H. Galbraith. 1983. Competition between fast- and slow-growing tropical legume rhizobia for nodulation of *Vigna unguiculata*. *Plant and Soil* 73:105-115.

Trotman, A. A., and R. W. Weaver. 1986. Number and effectiveness of cowpea rhizobia in soils of Guyana. *Tropical Agriculture* 63:129-132.

van Berkum, P., and B. B. Bohlool. 1980. Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses. *Microbiology Review* 44:491-517.

Viteri, S. E., and E. L. Schmidt. 1987. Ecology of indigenous soil rhizobia: Response of *Bradyrhizobium japonicum* to readily available substrates. *Applied and Environmental Microbiology* 53:1872-1875.

von Bulow, J. F. W., and J. Dobereiner. 1975. Potential for nitrogen fixation in maize genotypes in Brazil. *Proceedings of the National Academy of Science, USA* 72:2389-2393.

Weaver, R. W., and L. R. Frederick. 1974. Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. II. Field studies. *Agronomy Journal* 66:233-236.

Weaver, R. W., L. R. Frederick, and L. C. Dumenil. 1972. Effect of soybean cropping and soil properties on numbers of *Rhizobium japonicum* in Iowa soils. *Soil Science* 114:137-141.

We[illegible]h, L. F., L. V. Boone, C. G. Chambliss, A. T. Christiansen, D. L. Mulvaney, M. G. Oldham, and J. W. Pendleton. 1973. Soybean yields with direct and residual nitrogen fertilization. *Agronomy Journal* 65:547-550.

Whelan, A. M., and M. Alexander. 1986. Effects of low pH and high Al, Mn and Fe levels on the survival of *Rhizobium trifolii* and the nodulation of subterranean clover. *Plant and Soil* 92:363-371.

Whipps, J. M., and J. M. Lynch. 1983. Substrate flow and utilization in the rhizosphere of cereals. *New Phytology* 95:605-623.

Williams, L. E., and D. A. Phillips. 1983. Increased soybean productivity with a Rhizobium japonicum mutant. Crop Science 23:246-250.

Wollum, A. G., II, and D. K. Cassel. 1984. Spatial variability of Rhizobium japonicum in two North Carolina soils. Soil Science Society of America Journal 48:1082-1086.

Young, N. R., D. M. Hughes, and L. R. Mytton. 1986. The response of white clover to different strains of Rhizobium trifolii in hill land reseeding: A second trail. Plant and Soil 94:277-284.