Chapter 6

SOIL STRESS FACTORS INFLUENCING SYMBIOTIC NITROGEN FIXATION

M. J. SADOWSKY
Department of Soil, Water, and Climate, and The BioTechnology Institute, University of Minnesota, St. Paul, MN 55108, U.S.A.

1. INTRODUCTION

The soil environment is under a constant state of change and, as such, can be relatively stressful for both macro- and microorganisms. Fluctuations in pH, nutrient availability, temperature, and water status, among other factors, greatly influence the growth, survival, and metabolic activity of soil microorganisms and plants, and their ability to enter into symbiotic interactions. Despite this situation, soils represent one of Earth's most productive ecospheres, accounting for a majority of primary and successional productivity. Consequently, microbes, plants, and other soil inhabitants have evolved to adapt to the ever changing and often inhospitable soil environment. In this Chapter, I will discuss stress factors in soils that influence symbiotic nitrogen fixation and I will do so from the perspective of both the host plant and the microsymbiont. However, the reader should be aware that, whereas some stress factors simultaneously affect both symbiotic partners, *e.g.*, water stress, others may differentially influence each partner to a seemingly different degree by different mechanisms. Moreover, both plants and microbes have often adopted different strategies for dealing with these stress factors.

2. IMPORTANCE OF SYMBIOTIC NITROGEN FIXATION

Nitrogen (N) is one of the major limiting nutrients for most crop and other plant species (Newbould, 1989). Moreover, fixed-N acquisition and assimilation is second in importance only to photosynthesis for plant growth (Vance, 1998; Graham and Vance, 2000). From several indications and by many estimations, the

D. Werner and W. E. Newton (eds.), Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment, 89-112.

world will no doubt face severe food shortages in the not-too-distant future, in part due to excessive population growth and the negative environmental impact associated with this increased growth. Although Waggoner (1994) suggested that the Earth's population will reach nearly twice its current level of over 10 billion people by the year 2035, there is still some debate on when this will actually occur. However, there is little debate that this will indeed occur sometime in the not-too-distant future. In addition, populations in developing (and less developed) countries, which reside in the tropical and subtropical regions of Asia, Africa, and Latin America, may account for *ca.* 90% of the projected world population. Today, in tropical countries, plant materials provide *ca.* 80% of the caloric and dietary protein needs of individuals and this situation is not expected to change in the near future. In the fairly recent past, humans used *ca.* 10% of the total fixed carbon that is produced by plants through photosynthetic activity (Golley *et al.,*, 1992); today, humans use *ca.* 40% of that carbon. Moreover, it is estimated that, by 2030, humans will require *ca.* 80% of all photosynthetically-fixed carbon to meet their dietary requirements. Taking it as a given that enhanced agricultural production will require the utilization of large areas of land that are now considered to be either marginally productive or even non-arable, several alternate strategies are needed to meet these considerable and increasing human dietary needs in the future.

Many diverse biological associations contribute to N_2 fixation (BNF) in both soil and aquatic systems (Sprent, 1984). However, in most agricultural systems, the primary source of biologically-fixed N (*ca.* 80%) occurs *via* the symbiotic interactions of legumes and soil bacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Allorhizobium*, *Mesorhizobium*, and *Azorhizobium* (Sadowsky and Graham, 1998; Vance, 1998). The other 20% is contributed mainly by the actinorrhizal (*e.g.*, by *Frankia*) and *Anabena-Azolla* types of symbiotic interactions. It has been estimated that legumes provide approximately 35% of the worldwide protein intake and that *ca.* 250 million ha of legumes are currently grown world wide. These symbiotic partners together fix an astounding 90 Tg N per year (Kinzig and Socolow, 1994) which points to the obvious conclusion that enhancing the both the use and management of biologically-fixed N will result in huge environmental and economic benefits. To put this situation in some perspective, it has been estimated that *ca.* 288 Tg of fuel (at a cost of $30 billion U.S. annually) would be required to replace the N fixed by legumes with anhydrous ammonia produced by the Haber-Bosch process. For the U.S. alone, either decreasing or eliminating the use of synthetic N-fertilizers could save an estimated $1.0-4.5 billion annually (Tauer, 1989). This reduction in the amount of fuel consumed will also reduce undesirable impacts of the increased use of industrially-derived fix N.

3. SYMBIOTIC INTERACTION OF LEGUMES WITH RHIZOBIA

In order to properly discuss how environmental stress factors influence symbiotic nitrogen fixation, it is important to understand how the micro- and macro-symbionts interact at the cellular and molecular levels. In some instances, environmental perturbation independently influences the nodulation and nitrogen-fixation processes. Although, in this chapter, I only discuss this topic in broad terms, several recent review articles cover this area in much greater detail (Hirsch *et al.*, 2001; Roy *et al.*, 2002; Vitousek e*t al.*, 2002).

3.1. The Nitrogen-fixation Process

Environmental symbiotic N_2 fixation requires the coordinate interaction of two major classes of genes present in rhizobia, the *nif* genes and *fix* genes. Only the *nif* genes, which encode the molybdenum-based enzyme system, are found in rhizobia and they have structural and functional-relatedness to the N_2-fixation genes found in *Klebsiella pneumoniae*. The structural *nif* genes from taxonomically diverse microbes are nearly identical and function in a similar manner to encode nitrogenase (Ruvkin and Ausubel, 1980). A majority of the *nif* genes are plasmid borne in most rhizobia, but are located on the chromosome in the bradyrhizobia. Nitrogen fixation in symbionts and free-living microbes is catalyzed by nitrogenase, an enzyme complex encoded by the *nifDK* and *nifH* genes. Nitrogenase itself consists of a molybdenum-iron protein (MoFe), sometimes called component 1, and an iron-containing protein (Fe), component 2. The MoFe protein subunits are encoded by *nifK* and *nifD* and an FeMo-cofactor (called FeMoco) is required for activation of the MoFe protein. This cofactor is assembled through the activity of the *nifB,V,N,H* and *E* genes. The Fe-protein subunit is encoded by the *nifH* gene. The organization and complexity of *nif* genes in microorganisms varies tremendously (Downie 1998). For example, in the free-living *K. pneumoniae,* at least 20 *nif* genes are organized in about 8 operons (Dean and Jacobson 1992). In most systems, however, the regulation of all *nif* genes is controlled by NifA (a positive activator of transcription) and NifL (the negative regulator).

Environmentally, *nif*-gene expression is regulated by both O_2 and fixed-nitrogen levels (Merrick and Edwards, 1995). For example, elevated soil ammonia (NH_3 or NH_4^+) concentrations allow NifL to act as a negative controller of gene expression by preventing NifA acting as an activator. In addition, elevated O_2 concentrations inhibit FixL from activating the transcriptional activator FixJ, which in turn prevents increases in NifA. Because NifA is the transcriptional activator of all other *nif* genes, elevated O_2 results in a net decrease in the synthesis of nitrogenase and a decrease in, or abolition of, symbiotic N_2 fixation (Monson *et al.,* 1995).

In addition to the *nif* genes, many other microbial genes are involved in symbiotic nitrogen fixation, these are collectively referred to as *fix* genes. Moreover, several other genes in the microsymbiont, including those for exopolysaccharide (Leigh and Walker, 1994; Glazebrook and Walker, 1989), hydrogen uptake (Baginsky *et al.*, 2002), glutamine synthase (Carlson *et al.*, 1987), dicarboxylate transport (Finan *et al.*, 1983; Jiang *et al.*, 1989), nodulation efficiency (Sanjuan and Olivares, 1989), β-1,2-glucans (Breedveld and Miller, 1994), and lipopolysaccharides (Carlson *et al.*, 1987), either directly or indirectly influence symbiotic N_2 fixation. Excellent in-depth reviews of the regulation of nitrogen fixation in free-living and symbiotic bacteria can be found in Merrick and Edwards (1995), Dean and Jacobson (1992), and Patriarca *et al.* (2002).

3.2. The Nodulation Process

The infection and nodulation process involves an intimate interaction of micro- and macro-symbiont, and is mediated by a bidirectional molecular communication between both symbiotic partners. The rhizobia induce two types of nodules on legumes, either determinant or indeterminant nodules (Franssen *et al.*, 1992). The indeterminant nodules are most commonly formed on temperate legumes (pea, clover, and alfalfa) inoculated with the fast-growing nodule bacteria, whereas determinate nodules are normally induced by bradyrhizobia on tropical legumes, such as soybean and common bean. Rhizobia infect host plants, and induce root- or stem-nodules, using three fundamentally different mechanisms: (i) *via* root hairs (Kijne, 1992); (ii) entry through wounds, cracks, or lesions (Boogerd and van Rossum, 1997); or (iii) *via* cavities located around root primorida of adventitious roots (Boivin *et al.* 1997).

In the root-hair mode of infection, rhizobia attach, often perpendicular, to susceptible root hairs within minutes of inoculation. Subsequent penetration of root-hair cell walls by rhizobia leads to root-hair curling, usually within 6-18 hours of inoculation. Rhizobia are enclosed within a plant-derived infection thread within the root-hair cell and move down the root hair towards the root cortex. Cell division within the root cortex, ahead of the approaching infection thread, eventually leads to the production of nodule primordia (Kijne, 1992). The infection thread spreads among cells of the nodule primordium and rhizobia are released into the host cortex by endocytosis. Within the host cytoplasm, the rhizobia are surrounded by a host-derived peribacteroid membrane, compartmentalizing the rhizobia into a symbiosome. Over time, the nodules expand and are usually visible 6-18 days after inoculation. Although nodulation initially is often heaviest in the crown of the root, secondary nodules frequently appear on lateral roots as the early crown nodules begin to senesce. The number and size of nodules on each legume host is controlled by the genotype of both the host and rhizobial partner, by the efficiency of the symbiotic interaction, by the presence of existing nodules, and by environmental factors, such as soil-nitrogen level and soil-moisture status (Caetano-Annoles, 1997; Sagan and Gresshoff, 1996; Singleton and Stockinger, 1983).

4. NODULATION AND NITROGEN-FIXATION GENETICS IN THE RHIZOBIA AND BRADYRHIZOBIA

The majority of genetic studies on rhizobia and bradyrhiziobia have concentrated on the genetics and molecular biology of nodulation and N_2 fixation. Over the last 17 years, advances in molecular biology and genetics have helped elucidated a large number of genes having symbiotic functions. In the fast growing species, symbiosis-related genes tend to be clustered on one or several relatively large symbiotic plasmids (Broughton *et al.,* 1984; Hombrecher *et al.,* 1981; Kondorosi *et al.*, 1989), whereas in the bradyrhizobia, these genes are chromosomally located. The review articles listed below should be consulted for more detailed information on the genetics of nodulation and nitrogen fixation (Bladergroen and Spaink, 1998; Boivin *et al.*, 1997; Debelle *et al.,* 2001; Gualtieri and Bisseling, 2000; Pueppke, 1996; Schultze and Kondorosi, 1998; Spaink, 1995; Spaink, 2000; van der Drift *et al.*, 1998).

Many of the bacterial genes involved in the formation of nodules on legumes have been identified and a complete description of the function of many of these genes can be found in several recent articles (Bladergroen and Spaink, 1998; Debelle *et al.,* 2001; Niner and Hirsch, 1998; Pueppke, 1996). Taken together, more than 70 nodulation genes have been identified in rhizobia, although only a subset of these may be found in any single strain. Interestingly, despite this relatively large number, only a relatively few genes are required for nodulation of legumes (Göttfert, 1993; Long *et al.,* 1985; Long, 1989; van Rhijn and Vanderleyden, 1995). Nodulation genes can be divided three broad groups based on their relationship to host specificity; they are common nodulation genes, host-specific nodulation genes (hsn), and genotype specific nodulation (gsn) genes (Bachem *et al.,* 1986; Bassam *et al.,* 1986; Broughton *et al.,* 1984; Davis *et al.*, 1988; Djordjevic *et al.*, 1985; Heron *et al.*, 1989; Horvath *et al.*, 1986; Lewin *et al.,* 1987; Lie, 1978; Lewis-Henderson *et al.*,1991; Meinhardt *et al.*, 1993, Nieuwkoop *et al.*, 1987; Sadowsky *et al.,* 1991; Sadowsky *et al.*, 1995; Wijffelman *et al.,* 1985).

4.1. Environmental Influences on Nodulation Genes

As might be expected from the number of genes involved, the induction and repression of bacterial nodulation genes is under tight regulatory control and is a major factor influencing host specificity (Spaink *et al.*, 1987) and response to environmental variables. The *nodD* gene can be viewed as a global regulatory gene which, together with plant flavonoid-signal molecules, activates transcription of other inducible nodulation genes (Banfalvi *et al.*, 1988; Boundy-Mills *et al.*, 1994; Djordjevic *et al.,* 1987; Fellay *et al.,* 1995; Göttfert *et al.*, 1988; Innes *et al.*, 1985; Kosslak *et al.*, 1987; Long 1989; Long, 2001; Martinez and Palacios, 1990; Mulligan and Long, 1985; Olson *et al.*, 1985; Peters *et al.*, 1986; Price *et al.*, 1992; Sadowsky *et al.*, 1988; van Brussel *et al.*, 1990; Zaat *et al.*, 1987). The flavonoid *nod*-gene inducers are specific for a particular legume-*Rhizobium* interaction (Schlaman *et al.*, 1998) and their production is influenced by environmental variables, like plant fertility, pH (Hubac *et al.*, 1994), and Nod factors (Schmidt *et al.*, 1994). Repressor proteins also play a role in *nod*-gene regulation (Kondorosi *et al.*, 1988; Kondorosi *et al.*, 1989; Kondorosi *et al.*, 1991; Stacey *et al.*, 2002).

The *nod* genes of the microsymbiont are involved in the production of extracellular nodulation factors, called lipochitinoligosaccharides (LCOs) (Carlson *et al.*, 1993; 1994; Downie, 1998; Lerouge *et al.*, 1990; Pueppke, 1996). LCOs stimulate the plant to produce more *nod*-gene inducers, to deform root hairs on their respective host plant, and to initiate cell division in the root cortex (Banfalvi *et al.*, 1989; Faucher *et al.,* 1989; Lerouge *et al.,* 1990; Price *et al.*, 1992; Relic *et al.,* 1993; Schultze *et al.*, 1992; Spaink *et al.,* 1991; van Brussel *et al.*, 1990). Purified Nod factors have been shown to induce nodules on the specific host plant (Downie, 1998; Mergaert *et al.,* 1993; Relic *et al.,* 1993, Schultze *et al.,* 1992; Truchet *et al.,* 1991). The functions of *nod* genes and the basic structure of Nod factors for *B.*

japonicum and several species of the genus *Rhizobium* have been reviewed (Carlson *et al.*, 1993; Debelle *et al.*, 2001; D'Haeze and Holster, 2002; Downie, 1998; Sanjuan *et al.*, 1992). Recently, Endre and coworkers (2002) reported that a receptor kinase, NORK, in *Medicago sativa* is essential for Nod-factor perception in alfalfa and that the NORK system initiates a signal cascade, which leads to nodulation. The production of Nod factors by rhizobia is influenced by pH, temperature, and both phosphorus and nitrogen concentration (McKay and Djordjevic, 1993). Production and excretion of *nod* metabolites by *Rhizobium leguminosarum* bv. *trifolii* are disrupted by the same environmental factors that reduce nodulation in the field.

Although much has been learned about legume nodulation over the last 7 years using genetic and molecular methods, the genomic revolution will no doubt substantially increase our understanding of this process at a very rapid rate. To date, the complete genomic sequence of the symbiotic plasmid from *Rhizobium* sp. strain NGR-234 (see http://genome.imb-jena.de/other/cfreiber/ pNGR234a2.html), *S. meliloti* (see http://cmgm.stanford.edu/~mbarnett/genome.htm and http://sequence.toulouse.inra.fr/meliloti.html), and the genomes of *M. loti* (see http://www.kazusa.or.jp/en/database.html) and *B. japonicum* (see http://www.genome.clemson.edu /~twood/projects/brady.html and http://www.kazusa.or.jp/en/database.html, and http://www.kazusa.or.jp/rhizobase/Bradyrhizobium/index.html) have been determined. For further information, please also see volume 3 of this series entitled *Genomes and Genomics of Nitrogen-fixing Organisms*.

5. RHIZOBIA IN THE SOIL ENVIRONMENT

Rhizobia can exist in two fundamentally different modes. They can live in soils either as free-living saprophytic heterotrophs or as legume-host-specific nitrogen-fixing symbionts. This dual mode of existence gives rhizobia several distinct advantages with respect to survival and persistence over most other soil bacteria. The bulk soil that surrounds legumes contains relatively large numbers of rhizobia, often approaching 10^6 cells g^{-1} in soils of the American Midwest (Ellis *et al.*, 1984) and sometimes up to 10^8 cells g^{-1} (Bottomley, 1992). The growth of rhizobia in the rhizosphere may be stimulated by plant root exudates (Van Egeraat, 1975), although more research is needed in this area. Interestingly, Phillips *et al.* (1999) reported that rhizobia can also stimulate growth and respiration of leguminous plants.

Generally speaking, rhizobia in soils are associated with aggregates (Mendes and Bottomley, 1998; Postma *et al.*, 1990), which gives them some degree of protection from perturbations by environmental and biotic factors. However, the nodule environment affords the rhizobia a unique niche in which to multiply while being protected. Nodules can contain more than 10^{10} rhizobia g-1 (McDermott *et al.*, 1987). Nodule senescence at the end of the growing season leads to the release of a large number of rhizobia into soils. Numerous studies have shown that a legume host is not needed for persistence (saprophytic competence) of rhizobia in soils (Bottomley, 1992; Brunel *et al.*, 1988; Chatel *et al.*, 1968; Kucey and Hynes, 1989). Although nodule bacteria and bacteroids, after release into the environment, are often susceptible to osmotic and other soil stress factors (Sutton, 1983), many of the released rhizobia survive and persist in the soil as free-living, heterotrophic saprophytes for long periods until they again come into contact with susceptible legume host (Diatloff, 1977; Brunel *et al.*, 1988; Lindstrom *et al.*, 1990).

6. STRESS FACTORS IN THE SOIL ENVIRONMENT THAT INFLUENCE N_2 FIXATION

Virtually any environmental factor that negatively influences either the growth of rhizobia or the host plant itself has a dramatic impact on symbiotic N_2 fixation. These factors can independently negatively influence the nodulation process itself, and thereby indirectly affect nitrogen fixation, or directly influence plant growth and vigor during post-nodulation events and so affect the efficient functioning of the nitrogenase enzyme complex. To facilitate discussion, I have separated these fundamentally different sets of factors below. However, the reader should be aware that, in some instances, some factors simultaneously affect both rhizobia and the host plant.

6.1. Soil Water Content and Stress

Soil water influences the growth of soil microorganisms through processes of diffusion, mass flow, and nutrient concentration (Paul and Clark, 1988). Soil water is related to soil pore space, and soils containing larger pores and pore spaces retain less water. Thus, soil aggregates having smaller internal pore spaces are more favorable environments for the growth of rhizobia and most soil microbes (Papendick and Campbell, 1981; Turco and Sadowsky, 1995). Soil-water content also directly influences the growth of rhizosphere microorganisms, like rhizobia, by decreasing water activity below critical tolerance limits and indirectly by altering plant growth, root architecture, and exudations. Poor nodulation of legumes in arid soils is likely due to decreases in population levels of rhizobia during the dry season. However, the influence of soil-water activity on plant growth and vigor, and hence nodulation, should not be ignored.

Water activity (A_w) and water potential (ψ) values are parameters often used for describing water relations with respect to microorganisms (Harris, 1981). Rhizobia vary widely in their tolerance to water stress, and there is little apparent correlation to taxonomic criteria and phylogenetic relationships. Although bradyrhizobia have been suggested to be more resistant to water stress than the rhizobia (Bushby and Marshall, 1977), the reverse has also been reported (Mahler and Wollum, 1981). In solute-controlled water stress conditions, such as those controlled by salts, bacteria are able to grow and survive when Aw values are in the range of 0.76 - 0.99. This range corresponds to water potential (ψ) values of -15 to -350 bars. In rhizobia (and all bacteria), both cell membranes and walls play a pivotal role in tolerance to water-potential stress. Under conditions of elevated salt or other osmolytes, water stress is dominated by the movement of water in response to the ψ gradient (Harris, 1981). The total ψ of a microbial cell is due to the sum of the ψ from components of intermediary metabolism, and the intracellular accumulation of stress and compatible solutes. Many microbes, including rhizobia, accumulate compatible solutes, such as amino acids, salts, and betaines, as a means to equilibrate internal and external osmotic concentrations (Csonka, 1991; Csonka and Epstein, 1996).

Osmoregulation is a complex problem for rhizobia because they must be able to adapt to unfavorable and changing environmental conditions, as well as to osmotic-stress conditions associated with the infection process itself and life in the nodule. The detrimental effects of salt stress on inoculum viability, nodulation and nitrogen fixation have been reported for many *Rhizobium* spp. strains (Israel, 1988). Thus, elevated osmotic conditions can limit symbiosis by affecting survival and proliferation of rhizobia in the soil, inhibiting the infection process, or by directly affecting root-nodule function.

Rhizobia have evolved a variety of mechanisms for adapting to osmotic stress, mostly by the intracellular accumulation of inorganic and/or organic solutes. For example, *R. meliloti* overcomes osmotic stress-induced growth inhibition by accumulating compatible solutes, such as K^+, glutamate, proline, glycine betaine, proline betaine, trehalose, and the dipeptide, N-acetylglutaminylglutamine amide (Bernard e*t al.*, 1986; Botsford, 1984; Botsford, 1990; LeRudulier and Bouillard, 1983; Smith *et al.*, 1988; Boscari *et al.*, 2002). Some compatible solutes can be used as either nitrogen or carbon sources for growth, suggesting that their catabolism may be regulated to prevent degradation during osmotic stress (Smith and Smith, 1989).

6.2. Desiccation Tolerance

Some species of *Rhizobium* are also susceptible to non solute-mediated desiccation, referred to here as matric-mediated drought conditions. Species of *Rhizobium* differ in their susceptibility to the detrimental effects of desiccation in natural soils. Slow-growing rhizobia are generally thought to survive desiccation in a sandy soil better than fast-growing types (Bushby and Marshall, 1977; Bushby and Marshall, 1977a). Mahler and Wollum (1980; 1981) reported that moisture level was the dominant factor influencing both short- and long-term survival of *B. japonicum* strains inoculated into a loamy sand. Both soil type and temperature are important factors influencing survival of rhizobia in desiccated soils (Boumahdi *et al.*, 2001; Mahler and Wollum, 1980; Trotman and Weaver, 1995). There is no information available concerning the genetics and physiology of desiccation resistance in rhizobia, however, Boumahdi *et al.* (2001) reported that a reduction in medium Aw led to a decrease in unsaturation of cellular fatty acids in rhizobia (Boumahdi, *et al.*, 2001).

On addition to soils, rapid moisture loss is responsible for rhizobial death on the seed surface (Vincent *et al.*, 1962). In addition, and generally speaking, rhizobia are more resistant to soil-water deficit (drought) than the plant itself. Nevertheless, rhizobial strains that are superior under drought conditions have been reported (Athar and Johnson, 1996; Athar and Johnson, 1997; Hunt *et al*, 1981).

The impact of drought conditions on N_2 fixation is also due to direct influences on the plant partner (see reviews by Serraj *et al.,* 1999 and Hungria and Vargas, 2000). Soil-water deficit influences several aspects of the host legume, including but not limited to, nodule establishment, carbon and nitrogen metabolism, nitrogenase functioning, and photosynthetically-derived energy supply. Drought stress should be thought of as influencing the host in a global manner, rather than a collection of individual processes. Results from studies over the last 30 or so years have shown that the N_2-fixation process itself is more sensitive to drought conditions than gas exchange from leaves (Durrand *et al.*, 1987; Sinclair *et al.*, 1986), the accumulation of plant dry matter and carbon assimilation (Sinclair *et al.*, 1987; Wery *et al.*, 1994), photosynthesis, and nitrate assimilation (Purcell and King, 1996; Wery *et al.*, 1988). However, as discussed above, soil moisture also indirectly influences total plant N_2 fixation by decreasing nodule mass and number (Sangakkara *et al.*, 1996; Sinclair *et al.,* 1988). The decline in nodule number under drought conditions is most likely due to impacts on the infection process itself. Root-hair infections and infection-thread formation are negatively influenced by drought conditions (Sprent, 1971; Graham, 1992).

6.3. Nutrient Stress

As might be expected, soil nutrient status has a tremendous influence on the symbiosis, as well as on the independent growth and survival of both partners. It should be noted, however, that in some cases, nutrient stresses are indirectly caused by changes in soil matric potential or acidity, which limit nutrient bioavailability, rather than to the lack of the presence of nutrients *per se*. When considering nutrient limitations to symbiotic nitrogen fixation, one must clearly separate factors affecting growth of the host from those influencing the microbe or the symbiotic interaction. For example, acid and water stress causes alterations in root growth, which can indirectly affect both nodulation and nitrogen fixation. This effect is thought to be mediated by abscisic acid (Zhang *et al.*, 2001).

Stress conditions apparently increase requirements for essential elements, such as Ca^{2+}, P, and N, in both plants and microsymbionts (Beck and Munns, 1984; O'Hara *et al.*, 1989; Zahran, 1999). Ca^{2+} might, in some instances, offset the deleterious influence of low pH on root growth and ion uptake (Torimitsu *et al.*, 1985) and increase *nod*-gene induction and expression (Richardson *et al.*, 1988). Calcium deficiency, with or without the confounding influence of low pH, also affects attachment of rhizobia to root hairs (Caetano-Anolles *et al.*, 1989; Smit *et al.*, 1992), and nodulation and nodule development (Alva *et al.*, 1990). Lastly, a calcium-spiking phenomenon is initiated in root-hair cells of legumes by nodulation factors and rhizobia (Wais *et al.*, 2002), suggesting that Ca^{2+} plays a pivotal role in symbiotic interactions at the molecular level.

Phosphorous supply and availability remains a severe limitation to nitrogen fixation and symbiotic interactions. About 33% of the ariable land in the world is limited by P availability (Graham and Vance, 2000; Pereira and Bliss, 1989; Sanchez and Euhara, 1983). This situation is especially true in soils impacted by low pH. There are marked differences in rhizobial and plant requirements for P (Beck and Munns, 1985; Pereira and Bliss, 1989) with the slow-growers being more tolerant to low P than the fast-growing rhizobia (Beck and Munns, 1985). Nitrogen-fixing plants have an increased requirement for P over those receiving direct nitrogen fertilization, owing perhaps to nodule development and signal transduction

(Graham and Vance, 2000), and to P-lipids in the large number of bacterioids. Moreover, in white lupin, P deficiency leads to enhanced P acquisition by the formation of proteoid roots (Johnson *et al.*, 1996), changes in carbon metabolism, enhanced secretion of citrate and malate from roots, and release of a novel acid phosphatase into the rhizosphere proteoid roots (Gilbert *et al.*, 1999). Nodules themselves are sinks for P (Hart, 1989) and nodulation and N_2 fixation are influenced strongly by P availability (Leung and Bottomley, 1987; Singleton *et al.*, 1985; Saxena and Rewari, 1991). The rhizobia respond to P stress in a manner analogous to the host plant; that is, low P induces expression of genes involved in P acquisition (phosphatases, phosphate transporters) and acidification of the root zone (Al-Niemi *et al.*, 1997; Smart *et al.*, 1984; Torriani-Gorini *et al.*, 1987).

In addition to macro nutrients, the growth and persistence of rhizobia in soils is also influenced by several other nutritional factors (reviewed by Brockwell *et al.*, 1995 and Bottomley, 1992). The rhizobia are metabolically diverse and have been shown to use a variety of both plant- and soil-derived compounds for growth. Interestingly, some of the same compounds that support growth have also been shown to be chemotactic and induce *nod* genes (Parke and Ornston, 1986; Sadowsky and Graham, 1998). In addition, supplementation of soil and inoculants with gutamate, glycerol, and organic matter has been shown to enhance the survival and numbers of rhizobia in soils and increase both early nodulation and N_2 fixation (Rynne *et al.*, 1994). This result indicates that, although rhizobia can surely persist in soils, their efficacy can be enhanced by carbon addition, which suggests that they are C limited in the natural state.

6.4. Soil pH Stress

The influence of soil pH on the nodulation process has been extensively examined, in part due to the World's large number of acid soils (see reviews by Graham, 1992 and Hungria and Vargas, 2000). Worldwide, more than 1.5 Gha of acid soils limit agriculture production (Edwards *et al.*, 1991; Graham and Vance, 2000) and as much as 25% of the earth's croplands are impacted by problems associated with soil acidity (Munns, 1986). Brockwell *et al.* (1991) reported a nearly 10^{-3} decrease in the number of *S. meliloti* in soils with a $\mathrm{pH} < 6$ compared to those with a $\mathrm{pH} > 7.0$. As found for soil moisture, there is a range of effects of soil pH on rhizobia, but relatively few grow and survive well below pH values of 4.5-5.0 (Graham *et al.*, 1994; Vargas and Graham, 1988).

Competitive interactions have been shown to be influenced by soil pH. Generally speaking, the bradyrhizobia are more acid tolerant than the rhizobia (Brockwell *et al.*, 1991; Date and Halliday, 1979; Keyser and Munns, 1979; Sadowsky and Graham, 1998), although some strains of *R. tropici* are very acid tolerant (Graham *et al.*, 1992). The influence of soil pH on the behavior of rhizobia in soils can be dramatic. Generally, *S. fredii* is more competitive than *B. japonicum* in soybean nodulation in neutral soils in Spain, but *B. japonicum* USDA 110 outcompeted *S. fredii* in soil at pH 4.9 (Triplett and Sadowsky, 1992). Furthermore, despite the fact that *R. etli* is more competitive than *R. tropici* in nodule formation with beans (Chaverra and Graham, 1992; Martinez Romero and Rosenblueth, 1990), acidification of soils led to the replacement of *R. etli* by introduced, acid-tolerant *R. tropici* (Anyango *et al.*, 1995; Hungria *et al.*, 1997). However, the relationship between soil acidity, competitiveness, and the ability to survive in acid soils is not always straightforward and due to resistance to acidity. For example, Richardson and Simpson (1989) reported that many rhizobia from acid soils are sensitive to acidity. They suggested that soil microniches protect these rhizobia from extremes of soil pH. So, merely isolating a rhizobial strain from root nodules of plants grown in acid conditions does not guarantee that the isolated strain will be acid resistant (Graham, 1992). Furthermore, metals, such as Al, Cu, and Mn that become more soluble at lower pH, may also secondarily contribute to the inhibition of the growth and persistence of rhizobia in acid soils (Cooper *et al.*, 1983; Coventry and Evans, 1989; Hungria and Vargas, 2000; Lal, 1993; Reeve *et al.*, 2002). Moreover, soil Ca and P levels are also influenced by soil pH (Bell *et al.*, 1989; Munns, 1970) and may secondarily influence the growth and survival of rhizobia. Nevertheless, results from several studies have indicated that tolerance to acid conditions in rhizobia is often correlated to the strains ability to maintain an internal pH approaching neutrality (pH 7.2-7.5) (Graham *et al.*, 1994; Kashket, 1985; O'Hara *et al.*, 1989). This ability has been suggested to be due to proton exclusion (Graham, 1991), enhanced cytoplasmic-buffering capacity (Krulwich *et al.*, 1985), the presence of acid-shock responses (Bhagwat and Apte, 1989), the presence of glutathione (Riccillo *et al.*, 2000), the maintenance of elevated cellular potassium and glutamate concentrations (Aarons and Graham, 1991; Graham *et al.*, 1992), membrane permeability (Chen *et al.*, 1993), and calcium metabolism (Howieson *et al.*, 1992).

Although the microsymbiont appears more pH sensitive than the host partner (Hungria and Vargas, 2000), acidity also influences both the growth of the legume plant and the infection process (Munns, 1986). This effect is, in part, most likely due to both a disruption of signal exchange between macro- and micro-symbionts (Hungria and Stacey, 1997) and repression of nodulation genes and excretion of Nod factor in the rhizobia (Richardson *et al.*, 1988). Interestingly, nodulated legumes appear more sensitive to metal toxicity by Mn and Al than to their N-fed control counterparts (Hungria and Vargas, 2000).

Recently, using molecular techniques and proteomics, Glenn and colleagues have shown that rhizobial genes, such as *actA, actP, exoR, lpiA, actR, actS,* and *phrR*, are essential for growth at low pH (Dilworth *et al.*, 2001; Glenn *et al.*, 1999; Reeve *et al.*, 2002). Vinuesa and coworkers (2003), using a *Tn*5-mutagenesis approach, isolated and characterized pH-responsive genes, *lpiA* and *atvA*, from *Rhizobium* tropici CIAT899. Complementation analyses indicated that *atvA*, an ortholog of the *A. tumefaciens acvB* gene, is required for acid tolerance.

6.5. Soil Temperature

Temperature has a marked influence on survival and persistence of rhizobial strains in soils. For example, cowpea rhizobia from the hot dry Sahel-savannah of West Africa grow at 37°C, and more than 90% of the strains isolated from this region grew well to 40°C (Eaglesham *et al.*, 1991). The influence of temperature on rhizobia appears to be both strain and soil dependent. For example, *Bradyrhizobium* sp. (lupins) was less susceptible than *R. leguminosarum* bv. *trifolii* to high soil temperatures, but addition of montmorillonite and illite remediated this problem in sandy soils (Marshall, 1964). Soil temperature also greatly influences competition for nodulation (Kluson *et al.*, 1986; Triplett and Sadowsky, 1996). This effect may, in part, be due to a temperature-induced delay in nodulation or the restriction of nodules to the sub-surface region (Munns *et al.*, 1977).

Both temperature extremes need to be examined when considering the influence of soil temperature on the growth and survival of rhizobia, and the latter two parameters need to be separated. For example, whereas rhizobia isolated from temperate regions often survive at 4°C, little growth occurs at this temperature (Trinick, 1982). Nevertheless, there have been reports of growth of rhizobia from the Canadian high arctic at 5°C (Prévost *et al.*, 1987) and 10°C (Caudry-Reznick *et al.*, 1986). However, the symbiosis itself is sensitive to low temperatures; cooler root-zone temperatures limit nodulation and nitrogen fixation in the soybean-*B. japonicum* symbiosis (Lynch and Smith, 1994; Zhang *et al.*, 1995).

There are a few generalities that can be made. Although high soil temperatures may lead to death of many rhizobia isolated from temperate climates, strains from tropical region generally survive better at high soil temperatures. Nevertheless, Somasegaran *et al.* (1984) reported that incubation of inoculant strains at 37°C led to a gradual decline in population levels over an 8-week period. Some temperature-tolerant *Bradyrhizobium* sp. strains that nodulate cowpea in Nigeria have also been noted (Hartel and Alexander, 1984). In addition, temperature-tolerant strains of rhizobia can be either artificially (Karanja and Wood, 1988; Hartel and Alexander, 1984) or naturally selected for (Zahran *et al.*, 1994). However, excessive temperature shock has been shown to cure plasmids in fast-growing strains and some strains, which were isolated from high-temperature environments, have a Fix$^-$ phenotype (Hungria and Franco, 1993; Moawad and Beck, 1991). Effective high-temperature (40°C) tolerant rhizobia that are capable of nodulating and fixing nitrogen with *Phaseolus vulgaris* (Hungria *et al.*, 1993; Michiels *et al.*, 1994), *Acacia* (Zerhari *et al.*, 2000), and *Prosopis* (Kulkarni and Nautiyal, 1999) have also been reported. Although there have been several attempts to adapt rhizobia to higher temperatures for inoculation of legumes in tropical regions, in most cases, incubation of strains at elevated temperatures results in the loss of either infectivity or effectivity (Segovia *et al.,* 1991; Wilkins, 1967).

Relatively high-root temperature has also been shown to influence infection, N_2-fixation ability, and legume growth (Arayankoon *et al.*, 1990; Hungria and Franco, 1993; Kishinevsky *et al.*, 1992; Michiels *et al.*, 1994; Munevar and Wollum, 1982) and it has a strong influence on specific strain and cultivar interactions (Arayankoon *et al.*, 1990; Munevar and Wollum, 1982). It appears that every legume/*Rhizobium* combination has an optimum temperature relationship, which is around 30°C for clover and pea, between 35-40°C for soybean, peanut and cowpea, and between 25-30°C for common bean (Michiels *et al.*, 1994; Piha and Munns, 1987). Exposure of both symbiotic partners to temperature extremes much above or below these critical temperatures impairs infection, nodulation, nodule development, and general nodule functioning (Gibson, 1971; Roughley, 1970) as well as both plant growth and productivity. High soil tempratures also restrict nodulation to sub-surface regions where cooler temperatures prevail (Graham, 1991).

The physiological basis feor the temperature sensitivity of the symbiosis is most likely complex because many cellular functions in both host and microbe are affected by elevated and low temperature. Nevertheless, elevated temperature directly influences the production or release of *nod*-gene inducers from soybean and bean (Hungria and Stacey, 1997), it alters nodule functioning due to leghemoglobin synthesis, nitrogenase activity, and hydrogen evolution, and, in addition, hastens nodule senescence (Hungri and Vargas 2000). Although heat-shock proteins have been found in rhizobia (Aarons and Graham, 1991; Labidi *et al.*, 2000; Michiels *et al.*, 1994; Munchbach *et al.*, 1999) and heat stress alters the mobility of LPS (Zahran *et al.*, 1994), their direct role in either heat tolerance or sensitivity has not been demonstrated.

7. CONCLUDING REMARKS

Despite many decades of progress and the acquisition of a large amount of useful information, the physiological and molecular bases for the tolerance of legume-microbe symbiotic systems to environmental stress remains largely unknown and empirical in nature. Although understanding these processes was originally thought to be straightforward and tractable, we have learned that we now have more questions than answers. This situation is perhaps due to the fact that abiotic stresses independently and differentially influence the host legume, the rhizobia, and the symbiotic couple. So where do we go from here? Clearly, more work needs to be done on the underlying molecular bases for tolerance to stress factors in both legume and microbe.

Recent advances in the genomics and proetomics of macro- and micro-symbionts will accelerate progress in this area by providing a wealth of information on how both host and microbe respond to environmental perturbations. For example, proteome analysis has been used to investigate oxidative stress in the *Rhizobium etili-Phaseolus vulgaris* symbiosis (del Carmen Vargas *et al.*, 2003), to define bacterial genes involved in growth at low pH (Dilworth *et al.*, 2001; Glenn *et al.*, 1999; Reeve *et al.*, 2002), and to examine cultivar-specific interactions between *Rhizobium leguminosarum* bv. *trifolii* and subterranean clover (Morris and Djordjevic, 2001). Similarly, Saalbach and coworkers (2002) and Wienkoop and Saalbach (2003) have used proteome analysis to investigate the proteins in the pea and lotus peribacteroid membrane, respectively, and Mathesius *et al.* (2001) have established a root proteome reference map of *Medicago truncatula* that can be used with expressed sequence tag databases (Fedorova *et al.*, 2002; Lamblin *et al.*, 2003) to investigate molecular mechanisms of root symbioses in legumes. This type of global organismal information at the genomic and proteomic levels, however, now needs to be coupled to traditional plant breeding and microbial selection efforts in order to rapidly define and utilize microbial and host genetic loci that are involved in tolerance to a large number of environmental stresses.

REFERENCES

Aarons, S. R., and Graham, P. H. (1991). Response of *Rhizobium leguminosarum bv. phaseoli* to acidity. *Plant Soil, 134*, 145-151.

Al-Niemi, T. S., Kahn, M. L., and McDermott, T. R. (1997). P metabolism in the bean-*Rhizobium tropici* symbiosis. *Plant Physiol., 113*, 1233-1242.

Alva, A. K., Assher, C. J., and Edwards, D. G. (1990). Effect of solution pH, external calcium concentration, and aluminum activity on nodulation and early growth of cowpea. *Aust J. Agric. Res., 41*, 359-365.

Anyango, B., Wilson, J. K., Beynon, J. L., and Giller, K. E. (1995). Diversity of rhizobia nodulating *Phaseolus vulgaris* in two Kenyan soils with contrasting pHs. *Appl. Environ. Microbiol., 61*, 416-421.

Arayankoon, T., Schomberg, H. H., and Weaver, R. W. (1990). Nodulation and N_2 fixation of guar at high root temperature. *Plant Soil, 126*, 209-213.

Athar, M., and Johnson, D. A. (1996). Nodulation, biomass production, and nitrogen fixation in alfalfa under drought. *J. Plant Nutr., 19*, 185-199.

Athar, M., and Johnson, D. A. (1997). Effect of drought on the growth and survival of *Rhizobium meliloti* strains from Pakistan and Nepal. *J. Arid Environ*., *35*, 335-340.

Bachem, C. W., Banfalvi, Z., Kondorosi, E., Schell, J., and Kondorosi, A. (1986). Identification of host range determinants in the *Rhizobium* species MPIK3030. *Mol. Gen. Genet*., *203*, 42-48.

Baginsky, C., Brito, B., Imperial, J., Palacios, J.-M., and Ruiz-Argüeso, T. (2002). Diversity and evolution of hydrogenase systems in rhizobia. *Appl. Environ. Microbiol., 68*, 4915-4924.

Banfalvi, Z., and Kondorosi, A. (1989). Production of root hair deformation factors by *Rhizobium meliloti* nodulation genes in *Escherichia coli,* HsnD (*nodH*) is involved in plant host-specific modification of the nodABC factor. *J. Mol. Biol., 13*, 1-12.

Banfalvi, Z., Nieuwkoop, A., Schell, M., Best, L., and Stacey, G. (1988). Regulation of *nod* gene expression in *Bradyrhizobium japonicum. Mol. Gen. Genet., 214*, 420-424.

Bassam, B. J., Rolfe, B. G., and Djordjevic, M. A. (1986). *Macroptilium atropurpureum* (siratro) host specificity genes are linked to a *nodD*- like gene in the broad host range *Rhizobium* strain NGR234. *Mol. Gen. Genet*., *203*, 49-57.

Beck, D. P., and Munns, D. N. (1985). Effect of calcium on the phosphorus nutrition of *Rhizobium meliloti. Soil Sci. Soc. Am. J., 49*, 334-337.

Beck, D. P, and Munns, D. N. (1984). Phosphate nutrition of *Rhizobium* sp., *Appl. Environ. Microbiol., 47*, 278-282.

Bell, W., Edwards, D. G., and Asher, C. J. (1989). External calcium requirements for growth and nodulation of six tropical food legumes grown in flowing solution culture. *Aust. J. Agric. Res., 40*, 85-96.

Bernard, T., Pocard, J. A., Perroud, B. and LeRudulier, D. (1986). Variations in the response of salt-stressed *Rhizobium* strains to betaines. *Arch. Microbiol., 143*, 359-364.

Bhagwat, A. A., and Apte, S. K. (1989). Comparative analysis of proteins induced by heat shock salinity and osmotic stress in the nitrogen-fixing *Cyanobacterium anabaena*-sp strain. *J. Bacteriol., 171*, 5187-5189.

Bladergroen, M. R., and Spaink, H. P. (1998). Genes and signal molecules involved in the rhizobia-*Leguminoseae* symbiosis. *Curr. Opin. Plant Biol., 1*, 353-359.

Boivin C., Ndoye, I., Molouba, F., Delajudie, P., Dupuy, N., and Dreyfus, B. (1997). Stem nodulation in legumes - diversity, mechanisms, and unusual characteristics. *Critical Rev. Plant Sci., 16*, 1-30.

Boogerd, F. C., and van Rossum, D. (1997). Nodulation of groundnut by *Bradyrhizobium* - a simple infection process by crack entry. *FEMS Microbiol. Rev*., *21*, 5-27.

Boscari, A., Mandon, K., Dupont, L., Poggi, M.-C., and Le Rudulier, D. (2002). BetS is a major glycine betaine/proline betaine transporter required for early osmotic adjustment in *Sinorhizobium meliloti. J. Bacteriol., 184*, 2654-2663.

Botsford, J. L. (1984). Osmoregulation in *R.meliloti*: Inhibition of growth by salts. *Arch. Microbiol., 137*, 124-127.

Botsford, J. L. (1990). Osmoregulation in *R.meliloti*: Production of glutamic acid in response to osmotic stress. *Appl. Environ. Microbiol., 56*, 488-494.

Bottomley, P. (1992). Ecology of *Rhizobium* and *Bradyrhizobium*. In G. Stacey, R. H. Burris, and H. J. Evans (Eds.), *Biological Nitrogen Fixation* (pp. 292-347). New York: Chapman & Hall.

Boumahdi, M, Mary, P and Hornez, J. P. (2001). Changes in fatty acid composition and degree of unsaturation of (brady)rhizobia as a response to phases of growth, reduced water activities and mild desiccation. *Antonie Van Leeuwenhoek, 79*, 73-79.

Boundy-Mills, K. L., Kosslak, R. M., Tully, R. E., Pueppke, S. G., Lohrke, S. and Sadowsky, M. J. (1994). Induction of the *Rhizobium fredii* nod box-independent nodulation gene *nolJ* requires a functional *nodD1* gene. *Mol. Plant-Microbe Interact., 7*, 305-308.

Breedveld, M. W. and Miller, K. J. (1994). Cyclic beta-glucans of members of the family *Rhizobiaceae. Microbiol. Rev., 58*, 145-161.

Brockwell, J., Bottomley, P. J. and Thies, J. E. (1995). Manipulation of rhizobia microflora for improving legume productivity and soil fertility, a critical assessment. *Plant Soil, 174*, 143-180.

Brockwell, J., Pilka, A. and Holliday, R. A. (1991). Soil pH is the major determinant of the numbers of naturally-occurring *Rhizobium meliloti* in non-cultivated soils in New South Wales. *Aust. J. Exp. Agric., 31*, 211-219.

Broughton, W. J., Heycke, N., Meyer, Z. A. and Pankhurst, C. E. (1984). Plasmid linked *nif* and *nod* genes in fast-growing rhizobia that nodulate *Glycine max*, *Psophocarpus tetragonolobus* and *Vigna unguiculata. Proc. Natl. Acad. Sci. USA, 81*, 3093-3097.

Brunel, B., Cleyet-Marel, J. C., Normand, P., and Bardin, R. (1988). Stability of *Bradyrhizobium japonicum* inoculants after introduction into soil. *Appl. Environ. Microbiol., 54*, 2636-2642.

Bushby, H. V. A., and Marshall, K. C. (1977). Water status of rhizobia in relation to their susceptibility to desiccation and to their protection by montmorillonite, *J. Gen. Microbiol., 99*, 19-27.

Bushby, H. V. A., and Marshall, K. C. (1977a). Some factors affecting the survival of root-nodule bacteria on desiccation. *Soil Biol. Biochem., 9*, 143-147.

Caetano-Anolles, G., Lagares, A., and Favelukes, G. (1989). Adsorption of *Rhizobium meliloti* to alfalfa roots, dependence on divalent cations and pH. *Plant Soil,* 117, 67-74.

Caetano-Anolles, G. (1997). Molecular dissection and improvement of the nodule symbiosis in legumes. *Field Crops Res., 53*, 47-68.

Carlson, R. W., Sanjuan, J., Bhat, U. R., Glushka, J., Spaink, H. P., Wijfjes, A. H. M., *et al.* (1993). The structures and biological activities of the lipo-oligosaccharide nodulation signals produced by type I and II strains of *Bradyrhizobium japonicum. J. Biol. Chem., 268*, 18372-18381.

Carlson, R. W., Kalembasa, S., Turowski, D., Pachori, P., and Noel, K. D. (1987). Characterization of the lipopolysaccharide from a *Rhizobium phaseoli* mutant that is defective in infection thread development. *J. Bacteriol., 169*, 4923–4928.

Carlson, R. W., Price, N. P. J., and Stacey, G. (1994). The biosynthesis of rhizobial lipo-oligosaccharide nodulation signal molecules. *Mol. Plant Microbe Interact.,* 7, 684-695.

Caudry-Reznick, S., Prevost, D., and Schulman, H. M. (1986). Some properties of arctic rhizobia. *Arch. Microbiol., 146*, 12-18.

Chatel, D. L., Greenwood, R. M., and Parker, C. A. (1968). Saprophytic competence as an important characteristic in the selection of *Rhizobium* for inoculation. *Proc. IXth Intern. Cong. Soil Sci. Adelaide, 2*, 65-73.

Chaverra, M. H., and Graham, P. H. (1992). Cultivar variation in traits affecting early nodulation of common bean. *Crop Sci., 32*, 1432-1436.

Chen, H., Richardson, A. E., and Rolfe, B. G. (1993). Studies on the physiological and genetic basis of acid tolerance in *Rhizobium leguminosarum* bv. *Trifolii. Appl. Environ. Microbiol., 59*, 1798-1804.

Cooper, J. E., Wood, M., and Holding, A. J. (1983). The influence of soil acidity factors on rhizobia. In D. G. Jones and D. R. Davies (Eds.), *Temperate Legumes. Physiology, Genetics and Nodulation* (pp. 319-335). London: Pittman.

Coventry, D. R., and Evans, J. (1989). Symbiotic nitrogen fixation and soil acidity. In A. D. Robson (Ed.), *Soil Acidity and Plant Growth* (pp. 103-137). Sydney: Academic Press.

Csonka, L. N. (1991). Prokaryotic Osmoregulation: Genetics and Physiology. *Ann. Rev. Microbiol., 45*, 569-606.

Csonka, L. N., and Epstein, W. (1996). Osmoregulation. In R. Curtiss III, *et al.* (Eds.), *Escherichia coli and Salmonella: Cellular and Molecular Biology, 2nd Ed.* (pp. 1210-1223). Washington D.C.:American Society for Microbiology.

D'Haeze, W., and Holsters, M. (2002). Nod factor structures, responses, and perception during initiation of nodule development. *Glycobiol., 12*, 79R-105R.

Date, R. A., and Halliday, J. (1979). Selecting *Rhizobium* for acid, infertile soils of the tropics. *Nature (Lond.), 277*, 62-64.

Davis E. O., Evans I. J., and Johnston, A. W. B. (1988). Identification of *nod*X, a gene that allows *Rhizobium leguminosarum* biovar *viceae* strain TOM to nodulate Afghanistan peas. *Mol. Gen. Genet., 212*, 531-535.

Dean, D. R., and Jacobson, M. R. (1992). Biochemical Genetics of Nitrogenase. In G. Stacey, R. H. Burris, and H. J. Evans (Eds.), *Biological Nitrogen Fixation* (pp. 763-834). New York: Chapman and Hall.

Debelle, F., Moulin, L., Mangin, B., Denarie, J., and Boivin, C. (2001). Nod genes and Nod signals and the evolution of the *Rhizobium* legume symbiosis. *Acta Biochim. Pol., 48*, 359-65.

Del Carmen Vargas, M., Encarnacion, S., Davalos, A., Reyes-Perez, A., Mora, Y., Garcia-De Los Santos, A., Brom, S., and Mora, J. (2003). Only one catalase, *katG*, is detectable in *Rhizobium etli*, and is encoded along with the regulator OxyR on a plasmid replicon. *Microbiol., 149*, 1165-1176.

Diatloff, A. (1977). Ecological studies of root nodule bacteria introduced into field environments. 6. Antigenic and symbiotic stability in *Lotononis* rhizobia over a 12-year period. *Soil Biol. Biochem., 9*, 85-88.

Dilworth, M. J., Howieson, J. G., Reeve, W. G., Tiwari, R. P., and Glenn, A. R. (2001). Acid tolerance in legume root nodule bacteria and selecting for it, *Aust. J. Experimen. Agric., 41*, 435-446.

Djordjevic, M. A., Schofield, P. R., and Rolfe, B. G. (1985). *Tn*5 mutagenesis of *Rhizobium trifolii* host-specific nodulation genes results in mutants with altered host-range ability, *Mol. Gen. Genet., 200*, 463-471.

Djordjevic, M. A., Redmond, J. W., Batley, M., and Rolfe, B. G. (1987). Clovers secrete specific phenolic compounds which either stimulate or repress *nod* gene expression in *Rhizobium trifolii. EMBO J., 6*, 1173-1179.

Downie, J. A. (1998). The *Rhizobiaceae.* In H. P. Spaink, A. Kondorosi, and P. J. J. Hooykaas (Eds.), *Functions of Rhizobial Nodulation Genes* (pp. 387-402). Dordrecht: Kluwer Academic Publishers.

Durrand, J. L., Sheehy, J. E., and Minchin, F. R. (1987). Nitrogenase activity, photosynthesis, and nodule water potential in soybean plants experiencing water deprivation. *J. Exper. Bot., 38*, 311-321.

Eaglesham, A., Seaman, B., Ahmad, H., Hassouna, S., Ayanaba A. and Mulongoy, K. (1991). High temperature tolerant "cowpea" rhizobia. In A. H. Gibson and W. E. Newton (Eds.), *Current Perspectives in Nitrogen Fixation* (p. 356). Canberra: Austral. Acad. Sci.

Edwards, D. G., Sharifuddin, H. A. H., Yusoff, M. N. M., Grundon, N. J., Shamshuddin, J., and Norhayati, M. (1991). The management of soil acidity for sustainable crop production. In R. J. Wright *et al.* (Eds.), *Plant-Soil Interaction at Low* pH (pp. 383-396). Dordrecht: Kluwer Academic Publishers.

Ellis, W. R., Ham, G. E., and Schmidt, E. L. (1984). Persistence and recovery of *Rhizobium japonicum* inoculum in a field soil. *Agron. J., 76*, 573-576.

Endre, G., Kereszt, A., Kevei, Z., Mihacea, S., Kalo, P., and Kiss, G. B. (2002). A receptor kinase gene regulating symbiotic nodule development. *Nature, 417*, 962-966.

Faucher, C., Camut, S., Denarie, J., and Truchet, G. (1989). The *nodH* and *nodQ* host range genes of *Rhizobium meliloti* behave as avirulence genes in *R. legumiosarum bv viceae* and determine changes in the production of plant-specific extracellular signals. *Mol. Plant-Microbe Interact., 2*, 291-300.

Fedorova, M., van de Mortel, J., Matsumoto, P. A., Cho, J., Town, C. D., VandenBosch, K. A., *et al.* (2002). Genome-wide identification of nodule-specific transcripts in the model legume *Medicago truncatula. Plant Physiol., 130*, 519-537.

Fellay, R., Perret, X., Viprey, V., and Broughton, W. J. (1995). Organization of host-inducible transcripts on the symbiotic plasmid of *Rhizobium* sp. NGR234. *Molec. Microbiol., 16*, 657–667.

Finan, T. M., Wood, J. M., and Jordon, D. C. (1983). Symbiotic properties of C4-dicarboxylic acid transport mutants of *Rhizobium leguminosarum. J. Bacteriol., 154*, 1403–1413.

Franssen, H. J, Nap, J.-P., and Bisseling, T. (1992). Nodulins in root nodule development. In G. Stacey, R. H. Burris, and H. J. Evans (Eds.), *Biological Nitrogen Fixation* (pp. 598-624). New York: Chapman and Hall.

Gibson, A. H. (1971). Factors in the physical and biological environment affecting nodulation and nitrogen fixation by legumes. *Plant Soil (Spec. Vol.)*, 139–152.

Gilbert, G. A., Knight, J. D., Vance, C. P., and Allan, D. L. (1999). Acid phosphatase activity in phosphorus-deficient white lupin roots. *Plant Cell Environ., 22*, 801-810.

Glazebrook, J., and Walker, G. C. (1989). A novel exopolysaccharide can function in place of the calcofluor-binding exopolysaccharide in nodulation of alfalfa by *Rhizobium meliloti. Cell, 56*, 661–672.

Glenn, A. R., Reeve, W. G., Tiwari, R. P., and Dilworth, M. J. (1999). Acid tolerance in root nodule bacteria. *Novartis Found Symp., 221*, 112-126.

Golley, F., Baudry, J., Berry, R., Bornkamm, R., Dahlberg, K., Jansson, *et al.* (1992). What is the road to sustainability. *INTECOL Bull., 20*, 15-20.

Göttfert, M. (1993). Regulation and function of rhizobial nodulation genes. *FEMS Microbiol. Lett., 104*, 39-64.

Göttfert, M., Webber, J., and Hennecke, H. (1988). Induction of a *nodA-lacZ* fusion in *Bradyrhizobium japonicum* by an isoflavone. *J. Plant Physiol., 132*, 394-397.

Graham, P. H. (1992). Stress tolerance in *Rhizobium* and *Bradyrhizobium* and nodulation under adverse soil conditions. *Can. J. Microbiol., 38*, 475-484.

Graham, P. H., and Vance, C. P. (2000). Nitrogen fixation in perspective, an overview of research and extension needs. *Field Crops Res., 65*, 93-106.

Graham, P. H., Draeger, K. J., Ferrey, M. L., Conroy, M. J., Hammer, B. E., Martinez, E., *et al*. (1994). Acid pH tolerance in strains of *Rhizobium* and *Bradyrhizobium*, and initial studies on the basis for pH tolerance of *Rhizobium tropici* UMR1899. *Can. J. Microbiol., 40*, 198-207.

Gualtieri, G., and Bisseling, T. (2000). The evolution of nodulation. *Plant Mol Biol., 42*, 181-194.

Harris, R. F. (1981). Effect of water potential on microbial growth and activity. In *Water potential Relations in Soil Microbiology.* SSSA special publication No. 9, (p. 23). Madison, WI: Soil Science Society of America.

Hart, A. L. (1989). Nodule phosphorus and nodule ctivity in white clover. *N. Z. J. Agric. Res., 32*, 145-149.

Hartel, P. G., and Alexander, M. (1984). Temperature and desiccation tolerance of cowpea rhizobia. *Can. J. Microbiol., 30*, 820-823.

Heron D. S., Ersek, T., Krishan, H. B., and Pueppke, S. G. (1989). Nodulation mutants of *Rhizobium fredii* USDA 257. *Molec. Plant-Microbe Interact., 2*, 4-10.

Hirsch, A. M., Lum, M. R., and Downie, J. A. (2001). What makes the rhizobia-legume symbiosis so special? *Plant Physiol., 127*, 1484-1492.

Hombrecher, G., Brewin, N. J., and Johnston, A. W. B. (1981). Linkage of genes for nitrogenase and nodulation ability on plasmids in *Rhizobium leguminosarum* and *R. phaseoli. Mol. Gen. Genet., 182*, 133-136.

Horvath, B., Kondorosi, E., John, M., Schmidt, J., Torok, I, Gyorgypal, *et al.* (1986). Organization, structure, and symbiotic function of *Rhizobium meliloti* nodulation genes determining host specificity for alfalfa. *Cell, 46*, 335-343.

Howieson, J. G., Robson, A. D., and Abbott, L. K. (1992). Calcium modifies pH effects on the growth of acid-tolerant and acid-sensitive *Rhizobium meliloti. Aust. J. Agric. Res., 43*, 765-772.

Hubac, C., Ferran, J., Tremolieres, A., and Kondorosi, A. (1994). Luteolin uptake by *Rhizobium meliloti,* Evidence for several steps, including an active extrusion process. *Microbiol., 140*, 2769-2774.

Hungria, M., and Franco, A. A. (1993). Effects of high temperature on nodulation and nitrogen fixation by *Phaseolus vulgaris* (L.). *Plant Soil, 149*, 95-102.

Hungria, M., Franco, A. A., and Sprent, J. I. (1993). New sources of high-temperature tolerant rhizobia for *Phaseolus vulgaris* (L.). *Plant Soil, 149*, 103-109.

Hungria, M. and Stacey, G. (1997). Molecular signals exchanged between host plants and rhizobia, basic aspects and potential application in agriculture. *Soil Biol. Biochem., 29*, 519–830.

Hungria, M., Vargas, M. A. T., and Araujo, R. S. (1997). Fixação biológica do nitrogênio em feijoeiro. In M. A. T. Vargas and M. Hungria (Eds.), *Biologia dos Solos dos Cerrados* (pp. 189-295). Planaltina, Brazil: EMBRAPA-CPAC.

Hungria, M., and Vargas, M. A. T. (2000). Environmental factors affecting N_2 fixation in grain legumes in the tropics, with emphasis on Brazil. *Field Crops Res., 65*, 151-164.

Hunt, P. J., Wollum, A. G., and Matheny, T. A. (1981). Effects of soil water on *Rhizobium japonicum* infection nitrogen accumulation and yield in Bragg soybean. *Agric. J., 73*, 501-505.

Innes, R. W., Kuempel, P. L., Plazinski, J., Canter-Cremers, H., Rolfe, B. G., and Djordjevic, M. A. (1985). Plant factors induce expression of nodulation and host-range genes in *R. trifolii.Mol. Gen. Genet., 201*, 426-432.

Israel D. W., *et al.* (1988). Relative performance of *Rhizobium* and *Bradyrhizobium* strains under different environmental conditions. *ISI Atlas of Science; Animal and Plant Sciences*, pp. 95-99. Philadelphia, PA: Institute for Scientific Information.

Jiang, J., Gu, B. H., Albright, L. M., and Nixon, B. T. (1989). Conservation between coding and regulatory elements of *Rhizobium meliloti* and *Rhizobium leguminosarum dct* genes. *J. Bacteriol., 171*, 5244-5253.

Johnson, J. F., Vance, C. P., and Allan, D. L. (1996). Phosphorus deficiency in *Lupinus albus*, altered lateral root development and enhanced expression of phosphoenolpyruvate carboxylase. *Plant Physiol., 112*, 31-41.

Karanja, N. K., and Wood, M. (1988). Selecting Rhizobium phaseoli strains for use with beans (*Phaseolus vulgaris* L.) in Kenya. Tolerance of high temperature and antibiotic resistance. *Plant Soil, 112*, 15-22.

Kashket, E. (1985). The proton motive force in bacteria. A critical assessment of methods. *Ann. Rev. Microbiol., 39*, 219–242.

Keyser, H. H., and Munns, D. N. (1979). Effects of calcium, manganese and aluminum on growth of rhizobia in acid media. *Soil Sci. Soc. Amer. J., 43*, 500-503.

Kijne, J. W. (1992). The *Rhizobium* infection process. In G. Stacey, R. H. Burris, and H. J. Evans (Eds.), *Biological Nitrogen Fixation* (pp. 349-398). New York: Chapman & Hall.

Kinzig, A. P., and Socolow, R. H. (1994). Is nitrogen fertilizer use nearing a balance - reply. *Phys. Today, 47*, 24-35.

Kishinevsky, B. D., Sen, D., and Weaver, R. W. (1992). Effect of high root temperature on the *Bradyrhizobium*-peanut symbiosis. *Plant Soil, 143*, 275-282.

Kluson, R. A., Kenworthy, W. J., and Weber, D. F. (1986). Soil temperature effects on competitiveness and growth of *Rhizobium japonicum* and on *Rhizobium*-induced chlorosis of soybean. *Plant Soil, 95*, 202-207.

Kondorosi, E., Gyuris, J., Schmidt, J., John, M., Duda, E., Schell, J., and Kondorosi, A. (1988). Positive and negative control of nodulation genes in *Rhizobium meliloti* strain 41. In D. P. S. Verma and R. Palacios (Eds.), *Molecular Microbe-Plant Interactions* (p. 73). St. Paul, MN: APS Press.

Kondorosi, E., Gyuris, J., Schmidt, J., John, M., Duda, E., Hoffman, B., Schell, J., and Kondorosi, A. (1989). Positive and negative control of nod gene expression in *Rhizobium meliloti* is required for optimal nodulation. *EMBO. J., 8*, 1331-1340.

Kondorosi, E., Pierre, M., Cren, M., Haumann, U., Buire, M., Hoffman, B., Schell, J., and Kondorosi, A. (1991). Identification of *nolR*, a negatively transacting factor controlling the *nod* regulon in *Rhizobium meliloti. J. Mol. Biol., 222*, 885-896.

Kosslak, R. M., Bookland, R., Barkei, J., Paaren, H. E., and Applebaum, E. R. (1987). Induction of *Bradyrhizobium japonicum* common *nod* genes by isoflavones isolated from *Glycine max. Proc. Natl. Acad. Sci. USA, 84*, 7428-7432.

Krulwich, T. A., Agus, R., Schneir, M., and Guffanti, A. A. (1985). Buffering capacity of bacilli that grow at different pH ranges. *J. Bacteriol., 162*, 768-772.

Kucey, R. M. N., and Hynes, M. F. (1989). Populations of *Rhizobium leguminosarum* biovars *phaseoli* and *viciae* in fields after bean or pea in rotation with nonlegumes. *Can. J. Microbiol., 35*, 661-667.

Kulkarni, S., and Nautiyal, C. S. (1999). Characterization of high temperature-tolerant rhizobia isolated from *Prosopis juliflora* grown in alkaline soil. *J. Gen. Appl. Microbiol., 45*, 213-220.

Lal, R. (1993). The role of no-till farming in sustainable agriculture in the tropics, *Anais do I Encontro Latinoamericano sobre Plantio Direto na Pequena Propriedade, IAPAR* (pp. 29–62). 22–26 Novembro, Ponta Grossa, Brazil.

Labidi, M., Laberge, S., Vezina, L. P., and Antoun, H. (2000). The *dnaJ* (hsp40) locus in *Rhizobium leguminosarum bv. phaseoli* is required for the establishment of an effective symbiosis with *Phaseolus vulgaris. Mol. Plant-Microbe Interact., 13*, 1271-1274.

Lamblin, A. F., Crow, J. A., Johnson, J. E., Silverstein, K. A., Kunau, T. M., Kilian, A., *et al.* (2003). MtDB: A database for personalized data mining of the model legume *Medicago truncatula* transcriptome. *Nucl. Acids Res., 31*, 196-201.

Lerouge, P., Roche, P., Faucher, C., Maillet, F., Truchet, G., Prome, J. C., and Denarie, J. (1990). Symbiotic host specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signals. *Nature, 344*, 781-784.

Leung, K., and Bottomley, P. J. (1987). Influence of phosphate on the growth and nodulation characteristics of *Rhizobium trifolii. Appl. Environ. Microbiol., 53*, 2098-2105.

Lewin, A., *et al.* (1987). Multiple host-specificity loci of the broad host-range *Rhizobium* sp. NGR234 selected using the widely compatible legume *Vigna unguiculata. Plant Mol. Biol., 8*, 447-459.

Lewis-Henderson, W. R., and Djordjevic, M. A. (1991). A cultivar-specific interaction between *Rhizobium leguminosarum* biovar *trifolii* and subterranean clover is controlled by *nodM*, other bacterial cultivar specificity genes, and a single recessive host gene. *J. Bacteriol., 173*, 2791-2799.

Lie, T.A. (1978). Symbiotic specialization in pea plants. The requirement of specific *Rhizobium* strains for peas from Afghanistan. *Ann. Appl. Biol*., *88*, 462-465.

Leigh, J. A., and Walker, G. C. (1994). Exopolysaccharides of *Rhizobium:* Synthesis, regulation and symbiotic function. *Trends Genet., 10*, 63-67.

LeRudulier, D., and Bouillard, L. (1983). Glycine betaine, an osmotic effector in *K. pneumonniae* and other members of *Enterobacteriaceae. Appl. Environ. Microbiol., 46*, 152-159.

Lindstrom, K., Lipsanen, P., and Kaijalainen, S. (1990). Stability of markers used for identification of two *Rhizobium galegae* inoculant strains after five years in the field. *Appl. Environ. Microbiol., 56*, 444-450.

Long, S. R., Egelhoff, T., Fisher, R. F., Jacobs, T. W., and Mulligan, J. T. (1985). Fine structure studies of *R. meliloti nodDABC* genes. In H. J. Evans, P. J. Bottomley, and W. E. Newton (Eds.), *Nitrogen Fixation Research Progress* (p. 87-94). Boston: Martinus Nijhoff Publishers.

Long, S. R. (1989). *Rhizobium*-legume nodulation, life together in the underground. *Cell, 56*, 203-214.

Long, S. R. (2001). Genes and signals in the *Rhizobium*-legume symbiosis. *Plant Physiol., 125*, 69-72.

Lynch, D. H., and Smith, D. L. (1994). The effects of low root-zone temperature stress on 2 soybean (*Glycine max*) genotypes when combined with *Bradyrhizobium* strains of varying geographic origin. *Physiol. Plant., 90*, 105-113.

Mahler, R. L., and Wollum, A. G. (1980). Influence of water potential on the survival of rhizobia in Goldsboro loamy sand. *Soil Sci. Soc. Am. J., 44*, 988-992.

Mahler, R. L., and Wollum, A. G. (1981). The influence of soil water potential and soil texture on the survival of *Rhizobium japonicum* and *Rhizobium leguminosarum* isolates in the soil. *Soil Sci. Soc. Am. J., 45*, 761-766.

Marshall, K. C. (1964). Survival of root nodule bacteria in dry soils exposed to high temperatures. *Aust. J. Agric. Res., 15*, 273-281.

Martinez-Romero, E., and Rosenblueth, M. (1990). Increased bean *Phaseolus vulgaris* L. nodulation competitiveness of genetically modified strains. *Appl. Environ. Microbiol., 56*, 2384-2388.

Martinez, E., Romero, D., and Palacios, R. (1990). The *Rhizobium* genome, *Crit. Rev. Plant Sci.,* 9, 59-93.

Mary, P., Dupuy, N., Dolhembiremon, C., Delfives, C., and Tailliez, R. (1994). Differences among *Rhizobium meliloti* and *Bradyrhizobium japonicum* strains in tolerance to desiccation and storage at different relative humidities. *Soil Biol. Biochem., 26*, 1125-1132.

Mathesius, U., Keijzers, G., Natera, S. H., Weinman, J. J., Djordjevic, M. A., and Rolfe, B. G. (2001). Establishment of a root proteome reference map for the model legume *Medicago truncatula* using the expressed sequence tag database for peptide mass fingerprinting. *Proteomics, 1*, 1424-1440.

McDermott, T. R., Graham P. H., and Brandwein, D. M. (1987). Viability of *Bradyrhizobium japonicum* bacteroids. *Arch. Mikrobiol., 148*, 100-106.

McKay, I. A., and Djordjevic, M. A. (1993). Production and excretion of *nod* metabolites by *Rhizobium leguminosarum bv. trifolii* are disrupted by the same environmental factors that reduce nodulation in the field. *Appl. Envir. Microbiol., 59*, 3385-3392.

Meinhardt, L. W., Krishnan, H. B., Balatti, P. A., and Pueppke, S. G. (1993). Molecular cloning and characterization of a Sym-plasmid locus that regulates cultivar-specific nodulation of soybean by *Rhizobium fredii* USDA 257. *Mol Microbiol., 9*, 17-27.

Mendes I. C., and Bottomley, P. J. (1998). Distribution of a population of *Rhizobium leguminosarum* bv *trifolii* among different size classes of soil aggregates. *Appl. Environ. Microbiol., 64*, 970-975

Mergaert, P., van Montagu, M., Prome, J.-C., and Holsters, M. (1993). Three unusual modifications, a D-arabinosyl, an N-methyl, and a carbamoyl group, are present on the Nod factors of *Azorhizobium caulinodans* strain ORS571. *Proc. Natl. Acad. Sci. USA, 90*, 1551-1555.

Merrick, M. J., and Edwards, R. A. (1995). Nitrogen control in bacteria. *Microbiol. Rev., 59*, 604-622.

Michiels, J., Verreth, C., and Vanderleyden, J. (1994). Effects of temperature on bean-nodulating *Rhizobium* strains. *Appl. Environ. Microbiol., 60*, 1206-1212.

Moawad, H., and Beck, D. (1991). Some characteristics of *Rhizobium leguminosarum* isolates from uninoculated field-grown lentil. *Soil Biol. Biochem., 23*, 917-925.

Monson, E., Ditta, G., and Helinski, D. (1995). The oxygen sensor protein, FixL, of *Rhizobium meliloti*. Role of histidine residues in heme binding, phosphorylation, and signal transduction. *J. Biol. Chem., 270*, 5243-5250.

Morris, A. C., and Djordjevic, M. A. (2001). Proteome analysis of cultivar-specific interactions between *Rhizobium leguminosarum* biovar *trifolii* and subterranean clover cultivar Woogenellup. *Electrophoresis, 22*, 586-98.

Mulligan, J. T., and Long, S. R. (1985). Induction of *Rhizobium meliloti nodC* expression by plant exudate requires *nodD. Proc. Natl. Acad. Sci. USA, 82*, 6609-6613.

Munchbach, M., Nocker, A., and Narberhaus, F. (1999). Multiple small heat shock proteins in rhizobia. *J. Bacteriol., 181*, 83-90.

Munevar, F., and Wollum, A. G. (1982). Response of soybean plants to high root temperature as affected by plant cultivar and *Rhizobium* strain. *Agron. J., 74*, 138-142.

Munns, D. N. (1970). Nodulation of *Medicago sativa* in solution culture. V. Calcium and pH requirements during infection. *Plant Soil, 32*, 90-102.

Munns, D. N. (1986). Acid soils tolerance in legumes and rhizobia. *Adv. Plant Nutr., 2*, 63-91.

Munns, D. N., Fogle, V. W., and Hallock, B. G. (1977). Alfalfa root nodule distribution and inhibition of nitrogen fixation by heat. *Agron. J., 69*, 377-380.

Newbould, P. (1989). The use of nitrogen fertilizer in agriculture. Where do we go practically and ecologically? *Plant Soil, 115*, 297-311.

Nieuwkoop, A. J., Banfalvi, Z., Deshmane, N., Gerhold, D., Schell, M., Sirotkin, K., and Stacey, G. (1987). A locus encoding host range is linked to the common nodulation genes of *Bradyrhizobium japonicum. J. Bacteriol., 169*, 2631-2638.

Niner, B. M., and Hirsch, A. M. (1998). How many *Rhizobium* genes, in addition to *nod, nif/fix*, and *exo*, are needed for nodule development and function. *Symbiosis*, *24*, 51-102.

O'Hara, G. W., Goss, T. J., Dilworth, M. J., and Glenn, A. R. (1989). Maintenance of intracellular pH and acid tolerance in *Rhizobium meliloti. Appl. Environ. Microbiol., 55*, 1870-1876.

Olson, E. R., Sadowsky, M. J., and Verma, D. P. S. (1985). Identification of genes involved in the *Rhizobium*-legume symbiosis by Mu-dI(*kan, lac*)-generated transcription fusions. *Bio/Tech., 3*, 143-149.

Papendick, R. I., and Campbell, G. S. (1981). Theory and measurement of water potential in soil, organic materials, plants, seeds, and microorganisms. In J. F. Parr, W. R. Gardner, and L. F. Elliott (Eds.), *Water potential relations in soil microbiology: Proceedings of a symposium sponsored by Divisions S-1 and S-3 of the Soil Science Society of America* (pp. 1-22). Madison, WI: Soil Science Society of America.

Parke, D., and Ornston, L. N. (1986). Enzymes of the β-ketoadipate pathway are inducible in *Rhizobium* and *Agrobacterium*, and constitutive in *Bradyrhizobium* spp. *J. Bacteriol., 165*, 288-292.

Patriarca, E. J., Tate, R., and Iaccarino, M. (2002). Key role of bacterial NH_4^+ metabolism in *Rhizobium*-plant symbiosis. *Microbiol. Mol. Biol. Rev., 66*, 203-222.

Paul, E. A., and Clark, F. E. (1988). *Soil Microbiology and Biochemistry.* San Diego, CA: Academic Press.

Pereira, P. A. A., and Bliss. F. A. (1989). Selection of common bean (*Phaseolus vulgaris* L.) for N_2 fixation at different levels of available phosphorus under field and environmentally-controlled conditions. *Plant Soil, 115*, 75-82.

Peters, N. K., Frost, J. W., and Long, S. R. (1986). A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. *Science, 233*, 977-979.

Phillips, D. A., Joseph, C. M., Yang, G. P., Martinez-Romero, E., Sanborn, J. R., and Volpin, H. (1999). Identification of lumichrome as a *Sinorhizobium* enhancer of alfalfa root respiration and shoot growth. *Proc. Natl. Acad. Sci. USA, 96*, 12275-12280.

Piha, M. I., and Munns, D. N. (1987). Sensitivity of the common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature, *Plant Soil, 98*, 183-194.

Postma, J., Hok-a-Hin, C. H., and van Veen, J. A. (1990). Role of microniches in protecting introduced *Rhizobium leguminosarum* biovar *trifolii* against competition and predation in soil, *Appl. Environ. Microbiol., 56*, 495-502.

Prévost, D., Bordeleau, L. M., Caudry-Reznick, S., Schulman, H. M., and Antoun, H. (1987). Characteristics of rhizobia isolated from three legumes indigenous to the high arctic, *Astragalus alpinus*, *Oxytropis maydelliana*, and *Oxytropis arctobia. Plant Soil, 98*, 313-324.

Price, N. J. P., Relic, B., Talmont, F., Lewin, A., Prome, D., Pueppke, S. G., *et al.* (1992). Broad-host-range *Rhizobium species* strain NGR234 secretes a family of carbamoylated and fucosylated nodulation signals that are *O*-acylated or sulphated. *Mol. Microbiol., 6*, 3575-3584.

Pueppke, S. G. (1996). The genetic and biochemical basis for nodulation of legumes by rhizobia. C*rit. Rev. Biotechnol., 16*, 1-51.

Purcell, L. C., and King, C. A. (1996). Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. *J. Plant Nutrit., 19*, 969-993.

Reeve, W. G., Tiwari, R. P., Kale, N. B., Dilworth, M. J., and Glenn, A. R. (2002). ActP controls copper homeostasis in *Rhizobium leguminosarum* bv. *viciae* and *Sinorhizobium meliloti* preventing low pH-induced copper toxicity. *Mol. Microbiol., 43*, 981-991.

Relic, B., Talmont, F., Kopcinska, J., Golinowski, J. W., Prome, J.-C., and Broughton W. J. (1993). Biological activity of *Rhizobium* sp. NGR234 Nod factors on *Macroptilium atropurpureum. Mol. Plant-Microbe Interact., 6,* 764-774.

Riccillo, P. M., Muglia, C. I., de Bruijn, F. J., Roe, A. J., Booth, I. R., and Aguilar, O. M. (2000). Glutathione is involved in environmental stress responses in *Rhizobium tropici*, including acid tolerance. *J. Bacteriol., 182*, 1748-53.

Richardson, A. E., Simpson, R. J., Djordjevic, M. A., and Rolfe, B. G. (1988). Expression of nodulation genes in *Rhizobium leguminosarum* bv. *trifolii* is affected by low pH and by Ca^{2+} and Al ions. *Appl. Environ. Microbiol., 54*, 2541-2548.

Richardson, A. E., and Simpson, R. J. (1989). Acid-tolerance and symbiotic effectiveness of *Rhizobium trifolii* associated with a *Trifolium subterraneaum* L. based pasture growing in an acid soil. *Soil Biol. Biochem., 21*, 87-95.

Roughley, R. J. (1970). The influence of root temperature, *Rhizobium* strain and host selection on the structure and nitrogen-fixing efficiency of the root nodules of *Trifolium subterraneum. Ann. Bot., 34*, 631-646.

Roy, R. N., Misra, R. V., and Montanez, A. (2002). Decreasing reliance on mineral nitrogen--yet more food. *Ambio., 31*, 177-83.

Ruvkin, G. B., and Ausubel, F. M. (1980). Interspecies homology of nitrogenase genes. *Proc. Natl. Acad. Sci. USA,* 77, 191–195.

Rynne, F. G., Glenn, A. R., and Dilworth, M. J. (1994). Effect of mutations in aromatic catabolism on the persistence and competitiveness of *Rhizobium leguminosarum* bv. *Trifolii. Soil Biol. Biochem., 26*, 703-710.

Saalbach, G., Erik, P., and Wienkoop, S. (2002). Characterisation by proteomics of peribacteroid space and peribacteroid membrane preparations from pea (*Pisum sativum*) symbiosomes. *Proteomics, 2*, 325-337.

Sadowsky, M. J., Olson, E. R., Foster, V. E., Kosslak, R. M., and Verma, D. P. S. (1988). Two host-inducible genes of *Rhizobium fredii* and the characterization of the inducing compound. *J. Bacteriol., 170*, 171-178.

Sadowsky, M. J., Cregan, P. B., Gottfert, M., Sharma, A., Gerhold, D., Rodriguez-Quniones, F., *et al.* (1991). The *Bradyrhizobium japonicum nolA* gene and its involvement in the genotype-specific nodulation of soybeans. *Proc. Natl. Acad. Sci. USA, 88*, 637-641.

Sadowsky, M. J., and Graham, P. H. (1998). Soil Biology of the *Rhizobiaceae.* In H. P. Spaink, A. Kondorosi, and P. J. J. Hooykaas (Eds.), *The Rhizobiaceae* (pp. 155-172). Dordrecht: Kluwer Academic Publishers.

Sadowsky, M. J., Kosslak, R. M., Golinska, B., Madrzak, C. J., and Cregan, P. B. (1995). Restriction of nodulation by *B. japonicum* is mediated by factors present in the roots of *Glycine max. Appl. Environ. Microbiol., 61*, 832-836.

Sagan, M., and Gresshoff, P. M. (1996). Developmental mapping of nodulation events in pea (*Pisum sativum* L.) using supernodulating plant genotypes and bacterial variability reveals both plant and *Rhizobium* control of nodulation regulation. *Plant Sci., 117*, 167-179.

Sanchez, P. A., and Euhara, G. (1983). Management considerations for acid soils with high phosphorus fixation capacity. In F. E. Kharawuch *et al.* (Eds.). Madison, WI: American Society for Agronomy.

Sangakkara, U. R., Hartwig, U. A. and Nosberger, J. (1996). Soil moisture and potassium affect the performance of symbiotic nitrogen fixation in faba bean and common bean. *Plant Soil, 184*, 123-130.

Sanjuan, J., and Olivares, J. (1989). Implication of *nifA* in regulation of genes located on a *Rhizobium meliloti* cryptic plasmid that effect nodulation efficiency, *J. Bacteriol., 171*, 4154–4161.

Saxena, A. K., and Rewari. R. B. (1991). The influence of phosphate and zinc on growth, nodulation and mineral composition of chickpea (*Cicer arietinum* L.) under salt stress. *World J. Microbiol. Biotechnol., 7*, 202-205.

Schlaman, H. R. M., Phillips, D. A., and Kondorosi, E. (1998). Genetic organization and transcriptional regulation of rhizobial nodulation genes. In H. P. Spaink, A. Kondorosi, and P. J. J. Hooykaas (Eds.), *The Rhizobiacea* (pp. 351-386). Dordrecht: Kluwer Academic Publishers.

Schmidt, P. E., Broughton, W. J., and Werner, D. (1994). Nod factors of *Bradyrhizobium japonicum* and *Rhizobium* sp. NGR 234 induce flavonoid accumulation in soybean root exudates. *Molec. Plant-Microbe Interact., 7*, 384-390.

Schultze, M., and Kondorosi, A. (1998). Regulation of symbiotic root nodule development. *Annu. Rev. Genet., 32*, 33-57.

Schultze, M., Quiclet-Sire, B., Kondorosi, E., Virelizier, H., Glushka, N., Endre, G., *et al.* (1992). *Rhizobium meliloti* produces a family of sulphated lipooligosaccharides exhibiting different degrees of plant host specificity. *Proc. Natl. Acad. Sci. USA, 89*, 192-196.

Segovia, L., Pinero, D., Palacios, R., and Martinez-Romero, E. (1991). Genetic structure of a soil population of nonsymbiotic *Rhizobium leguminosarum. Appl. Environ. Microbiol., 57*, 426-43.

Serraj, R. and Sinclair, T. R. (1997). Variation among soybean cultivars in dinitrogen fixation response to drought. *Agron. J., 89*, 963-969.

Serraj, R., Sinclair, T., and Purcell, L. (1999). Symbiotic N_2 fixation response to drought. *J. Exp. Bot., 50*, 143-155.

Sinclair, T. R. (1986). Water and nitrogen limitation in soybean grain production. I. Model development. *Field Crops Res., 15*, 125-141.

Sinclair, T. R., Muchow, R. C., Bennett, J. M., and Hammond, L. C. (1987). Relative sensitivity of nitrogen and biomass accumulation to drought in field-grown soybean. *Agron. J., 79*, 986-991.

Sinclair, T. R., Zimet, A. R., and Muchow, R. C. (1988). Changes in soybean nodule number and dry weight in response to drought. *Field Crops Res., 18*, 197-202.

Singleton, P. W., and Stockinger, K. R. (1983). Compensation against ineffective nodulation in soybean (*Glycine max*). *Crop Sci., 23*, 69-72.

Singleton, P. W., Abel Magid, H. M., and Tavares, J. W. (1985). Effect of phosphorus on the effectiveness of strains of *Rhizobium japonicum. Soil Sci. Soc. Am. J., 49*, 613-616.

Smart, J. B., Dilworth, M. J., and Robson, A. D. (1984). A continuous culture study of the phosphorus nutrition of *Rhizobium trifolii* WU95, *Rhizobium* NGR234 and *Bradyrhizobium* CB756. *Arch. Microbiol., 140*, 276-280.

Smit, G., Swart, S., Lugtenberg, B. J. J., and Kijne, J. W. (1992). Molecular mechanisms of attachment of *Rhizobium* bacteria to plant roots. *Mol. Microbiol., 6*, 2897-2903.

Smith, L., and Smith, G. M. (1989). An osmoregulated dipeptitide in stressed *R. meliloti. J. Bacteriol., 171*, 4714-4717.

Smith, L. T., Pocard, J. A., Bernard, T., and LeRudulier, D. (1988). Osmotic control of glycine betaine biosynthesis and degradation in *R. melilot. J. Bacteriol., 170*, 3142-3149.

Somasegaran, P., Reyes, V. G., and Hoben, H. J. (1984). The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage and distribution. *Can. J. Microbiol., 30*, 23-30.

Spaink, H. P., Sheeley, D. M., van Brussel, A. A. N., Glushka, J., York, W. S., Tak, T., *et al.* (1991). A novel highly unsaturated fatty acid moiety of lipooligosaccharide signals determines host specificity of *Rhizobium. Nature, 354*, 124-130.

Spaink, H. P. (1995). The molecular basis of infection and nodulation by rhizobia - the ins and outs of sympathogenesis. *Ann. Rev. Phytopath., 33*, 345–368.

Spaink, H. P. (2000). Root nodulation and infection factors produced by rhizobial bacteria. *Annu. Rev. Microbiol., 54*, 257-288.

Spaink, H. P., Wijffelman, C. A, Pees, E., Okker, R. J. H., and Lugtenberg, B. J. J. (1987). *Rhizobium* nodulation gene *nodD* as a determinant of host specificity. *Nature, 328*, 337-340.

Sprent, J. I. (1971). Effects of water stress on nitrogen fixation in root nodules. *Plant Soil (Spec. Vol.),* 225–228.

Sprent, J. I. (1984). Nitrogen fixation. In M. B. Wilkins (Ed.), *Advances in Plant Physiology* (pp. 249-276). London: Pitman.

Stacey, G., *et al.* (2002). Signal exchange during the early events of soybean nodulation. In T. M. Finan, M. R. O'Brian, D. B. Layzell, J. K. Vessey, and W. E. Newton (Eds.), *Nitrogen Fixation Global Perspectives* (pp. 118-122). Wallingford, UK: CABI Publishing.

Sutton, W. D. (1983). Nodule development and senescence. In W. J. Broughton (Ed.), *Nitrogen Fixation, Vol. 3 Legumes* (pp. 144-212). Oxford, UK: Clarendon Press.

Tauer, L. (1989). Economic impact of future biological nitrogen fixation technologies on United States Agriculture. *Plant Soil, 119*, 261-270.

Torimitsu, K., Hayashi, M., Ohta, E., and Sakata, M. (1985). Effect of K^+ and H^+ stress and role of Ca^{2+} in the regulation of intracellular K^+ concentration in mung bean roots. *Physiol. Plant., 63*, 247-252.

Torriani-Gorini, A., Rothman, F. G., Silver, S., *et al.* (1987). *Phosphate metabolism and cellular regulation in microorganisms*. Washington, D.C.: American Society for Microbiology.

Trinick, M. J. (1982). Biology. In W. J. Broughton (Ed.), *Nitrogen Fixation, Vol. 2* Rhizobium (pp. 76-146). Oxford, UK: Clarendon Press.

Triplett, E. W., and Sadowsky, M. J. (1992). Genetics of competition for nodulation. *Ann. Rev. Microbiol., 46*, 399-428.

Trotman, A. P., and Weaver, R. W. (1995). Tolerance of clover rhizobia to heat and desiccation stresses in soil. *Soil Sci. Soc. Am. J., 59*, 466-470.

Truchet, G., Roche, P., Lerouge, P., Vasse, J., and Camut, S. (1991). Sulphated lipo-oligosaccharide signals of the symbiotic procaryote *Rhizobium meliloti* elicit root nodule organogenesis on the host plant *Medicago sativa. Nature, 351*, 670-673.

Turco, R. F., and Sadowsky, M. J. (1995). Understanding the microflora of bioremediation. In H. D. Skipper and R. F. Turco (Eds.), Bioremediation: Science and Applications. *Soil Science (Special Publication) 43* (pp. 87-103). Madison, WI: Soil Science Society of America.

van Brussel, A., Recourt, K., Pees, E., Spaink, H. P., Tak, T., Wijffelman, C., Kijne, J. W., and Lugtenberg, B. J. J. (1990). A biovar specific signal of *Rhizobium leguminosarum* bv. *viceae* induces increased nodulation gene-inducing activity in root exudate of *Vicia sativa* subsp. *nigra. J. Bacteriol., 172*, 5394-5401.

van der Drift, K. M. G. M, Olsthoorn, M. M. A. L., Brull, P., Blok-Tip, L., and Thomas-Oates, J. E. (1998). Mass spectrometric analysis of lipo-chitin oligosaccharide signal molecules mediating the host-specific legume-*Rhizobium* symbiosis. *Mass Spec. Rev., 17*, 75–95.

Van Egeraat, A. W. S. M. (1975). The possible role of homoserine in the development of *Rhizobium leguminosarum* in the rhizosphere of pea seedlings. *Plant Soil, 42*, 381-386.

van Rhijn, P., and Vanderleyden, J. (1995). The *Rhizobium*-plant symbiosis, *Microbiol. Rev., 59*, 124-142.

Vance, C. P. (1998). Legume symbiotic nitrogen fixation, agronomic aspects. In H. P. Spaink, A. Kondorosi, and P. J. J. Hooykaas (Eds.), *The Rhizobiaceae* (pp. 509-530). Dordrecht: Kluwer Academic Publishers.

Vargas, A. A. T., and Graham, P. H. (1988). *Phaseolus vulgaris* cultivar and *Rhizobium* strain variation in acid-pH tolerance and nodulation under acid conditions. *Field Crops Res., 19*, 91-101.

Vincent, J. M, Thompson, J. A., and Donovan, K. O. (1962). Death of root nodule bacteria on drying. *Australian J. Agric. Res., 13*, 258-270.

Vinuesa, P., Neumann-Silkow, F., Pacios-Bras, Ch., Spaink, H. P., Martinez-Romero, E., and Werner, D. (2003). Genetic analysis of a pH regulated operon from *Rhizobium tropici* CIAT 899 involved in acid tolerance and nodulation competitiveness, *Mol. Plant-Microbe Interact., 16*, 159-168.

Vitousek, P. M., Hattenschwiler, S., Olander, L. and Allison, S. (2002). Nitrogen and nature. *Ambio., 31*, 97-101.

Waggoner, P. E. (1994). How much land can ten million people spare for nature? *Task Force Report 121* (p. 64). Ames, IA: Council on Agricultural Science and Technology.

Wais, R. J., Keating, D. H., and Long, S. R. (2002). Structure-function analysis of *nod* factor-induced root hair calcium spiking in *Rhizobium*-legume symbiosis, *Plant Physiol., 129*, 211-224.

Wery, J., Deshamps, M., and Leger-Cresson, N. (1988). Influence of some agro-climatic factors and agronomic practices on nitrogen nutrition of chickpea. In D. P. Beck and L. A. Materon (Eds.), *Nitrogen fixation by legumes in Mediterranean agriculture* (pp. 287-301). Dordrecht: ICARDA-Martinus Nijhoff.

Wery, J., Silim, S. N., Knight, E. J., Malhorta, R. S., and Cousin, R. (1994). Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes. *Euphytica, 70*, 487-495.

Wienkoop, S., and Saalbach, G. (2003). Proteome analysis. Novel proteins identified at the peribacteroid membrane from *Lotus japonicus* root nodules. *Plant Physiol., 131*, 1080-1090.

Wijffelman, C. A., Pees, E., van Brussel, A. A., Priem, M., Okker, R., and Lugtenberg, B. J. J. (1985). Analysis of the nodulation region of the *Rhizobium leguminosarum* Sym plasmid pRL1JI. In H. J. Evans, P. J. Bottomly, and W. E. Newton (Eds.), *Nitrogen Fixation Research Progress* (pp. 127). Boston, MA: Martinus Nijhoff Publishers.

Wilkins, J. (1967). The effects of high temperature on certain root-nodule bacteria. *Aust. J. Agric. Res., 18*, 299-304.

Zaat, S. A. J., Wijffelman, C. A., Spaink, H. P., van Brussel, A. A. N., Okker, R. J. H., and Lugtenberg, B. J. J. (1987). Induction of the *nodA* promoter of *Rhizobium leguminosarum* Sym plasmid pRL1JI by plant flavanones and flavones. *J. Bacteriol., 169*, 198–204.

Zahran, H. H. (1999). *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in arid climate, *Microbiol. Molec. Biol. Rev.,* 63*,* 968-989.

Zahran, H. H., Rasanen, L. A., Karsisto, M., and Lindstrom, K. (1994). Alteration of lipopolysaccharide and protein profiles in SDS-PAGE of rhizobia by osmotic and heat stress. *World J. Microbiol. Biotechnol., 10*, 100-105.

Zerhari, K., Aurag, J., Khbaya, B., Kharchaf, D., and Filali-Mattouf, A. (2000). Phenotypic characteristics of rhizobia isolates nodulating *Acacia* species in the arid and Saharan regions of Morocco. *Lett. Appl. Microbiol., 30*, 351-357.

Zhang, F., Lynch, D. H., and Smith, D. L. (1995). Impact of low root temperature in soybean [*Glycine max* (L. Merr.)] on nodulation and nitrogen fixation. *Environ. Exper. Bot., 35*, 279-285.

Zhang, S. Q., Outlaw, W. H., Jr., and Aghoram, K. (2001). Relationship between changes in the guard cell abscisic-acid content and other stress-related physiological parameters in intact plants, *J. Exp. Bot., 52*, 301-308.