# 8 Legume Response to Rhizobial Inoculation in the Tropics: Myths and Realities

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On the global scale, symbiotic $N_2$-fixing legumes contribute a major source of N to the biosphere, though estimates of the actual amounts vary considerably (e.g., see Evans & Barber, 1977). In the tropics, legumes are an integral part of forest ecosystems, natural pastures, and agricultural systems. Legume biological $N_2$ fixation (BNF) offers an especially attractive alternative to fossil fuel-derived N fertilizers in subsistence farming and sustainable agricultural programs in developing countries where hard currency is in short supply and fertilizer N is virtually unavailable on the local markets.

In the USA, Canada, Australia, and other developed countries, demonstration of benefits from inoculating certain legumes has led to the formation of commercial enterprises based on the inoculant production technology. In the developing tropics, however, promotion and use of rhizobial inoculants and the establishment of an inoculant industry would be successful only if significant benefits could be demonstrated for important tropical legumes.

Inoculation success/failure is highly site specific. It depends on such a multitude of interacting factors that it would be virtually impossible to make generalizations based on data gathered by different workers without standardization. Despite these difficulties, Vincent (1982) has done an admirable job of compiling information gathered from scientists from South and Southeast Asia participating in a workshop, Nitrogen Fixation by Legumes for Tropical Agriculture. Much of the information is sketchy and incomplete, but as the author points out ". . .together they give a useful insight into the significance of legumes, needs associated with their successful utilization and some indications of potential for an improved contribution."

Nutman (1976) describes a series of inoculation trials conducted under the organization of the International Biology Program (IBP) during the late 1960s and early 1970s. The design of these experiments included measures of factors controlling the response to inoculation of alfalfa (*Medicago sativa* L.) in numerous locations. While the benefit of inoculating alfalfa was clearly demonstrated in these experiments, there has not been until recent

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years a comprehensive effort to evaluate the response to rhizobial inoculation of tropical legumes. Given the widespread misconceptions about tropical rhizobia and the need for farmers in the tropics to inoculate their legume crops, it is imperative that reliable data on the benefits from inoculation be available.

## THE MYTH OF THE PROMISCUITY OF TROPICAL LEGUMES

Legumes require the aid of their symbiotic nodule-forming bacterial partners, rhizobia, for $N_2$ fixation. Not all rhizobia are capable of forming nodules on all legumes. There are host-bacterial specificities expressed by both partners. The so-called "cross-inoculation" groups are comprised of groups of legumes that nodulate with the same group of bacteria, which are then classified together in the same bacterial species. Some cross-inoculation groups are highly specific and contain a limited number of legume species, while others are more promiscuous and consist of a wide range of legumes. From the bacterial perspective, some species are highly selective and others have a broader host range. This host range serves the basis for the classification of the root nodule bacteria given in Table 8-1.

The genus *Rhizobium* contains all of the "fast-growing" acid-producing bacteria, while the genus *Bradyrhizobium* contains those that are slow growing and do not produce acid on yeast-mannitol media. The categories *R. loti* and *Bradyrhizobium* spp. are basically "dumping-grounds" for groups of related and not-so-related bacteria whose host ranges are not fully known. The *Bradyrhizobium* spp. or "cowpea miscellany," for example, are purported to contain the rhizobia that nodulate tropical legumes, and here lies

Table 8-1. Summary of recent modifications to rhizobial classification: Family Rhizobiaceae.†

| Bacteria | Host legume |
|---|---|
| Genus: *Rhizobium* | |
| *R. leguminosarum* | |
| bv. *viceae* | *Vicia/Pisum/Lens/Lathyrus* |
| bv. *trifolii* | *Trifolium* spp. (with exceptions) |
| bv. *phaseoli* | *Phaseolus* spp. (temperate species *vulgaris, angustifolius, coccineus*) |
| *R. meliloti* | *Melilotus/Medicago/Trigonella* |
| *R. loti* | *Lotus/Lupinus/Cicer/Anthyllis/Leucaena* and many other tropical tree legumes |
| *R. fredii*‡ | *Glycine* spp. |
| Genus: *Azorhizobium* | |
| *A. caulinodans* | *Sesbania rostrata* (stem nodules) |
| Genus: *Bradyrhizobium* | |
| *B. japonicum* | *Glycine* spp. (*G. max, G. soja*) |
| *Bradyrhizobium* sp. | Many tropical legumes |

† Compiled from Dreyfus et al. (1988), Jordan (1984), Scholla and Elkan (1984).
‡ It has recently been proposed that the *R. fredii* group be renamed *Sinorhizobium fredii* comb. nov. and, along with *S. xinjiangensis* sp. nov., be placed in a separate genus *Sinorhizobium* gen. nov. (Chen et al., 1988).

the basis for the myth that because of their promiscuity, tropical legumes do not respond to inoculation in the field.

What is most important to recognize in dealing with this myth is that taxonomic units are defined by scientists for their own convenience. Also, bacteria do not recognize taxonomic boundaries; they are limited only by their own genetic compositions. The limitations of the taxonomic classifications are underscored by the fact that there are cross-overs in terms of host nodulation between bacteria in different species as well as genera. For example, it is now well known that soybean [*Glycine max* (L.) Merr.], lupin (*Lupinus* spp.), lotus (*Lotus* spp.), and others can be nodulated by bacteria in both the *Rhizobium* and *Bradyrhizobium* genera.

It is equally important to recognize that within each "infectivity" grouping there are both effectivity and infectivity subgroups. Not all bacterial strains nodulating a group of legumes are effective in $N_2$ fixation on all the species. An example is the case of *R. leguminosarum* bv. *trifolii* that nodulate clover species. Not all strains that are highly effective on red clover (*Trifolium pratense* L.) and white clover (*T. repens*) are necessarily effective on cultivars of subclover (*T. subterraneum* L.) (Gibson et al., 1975). Burton (1979) compiled a comprehensive list of "effectiveness groupings" for several agriculturally important legumes within cross-inoculation groups. But, he cautions that not all plants in an effectiveness group will respond similarly to all the rhizobial strains.

Graham and Hubbell (1975) have grouped tropical legumes into three broad categories based on their nodulation and $N_2$-fixation specificity. The group containing *Vigna, Calopogonium, Arachis, Dolichos, Glycine wightii*, and others are promiscuous and nodulate with an array of bacteria isolated from a wide range of legumes. The second group contains legumes that are highly specific in their rhizobial requirement. Such legumes as *Trifolium semipilosum* Fresen., *Glycine max (L.), Leucaena leucocephala* (Lam.), and *Lotononis bainesii* are placed in this group. Intermediate between the two extremes is the third group of tropical legumes that nodulate with a wide range of rhizobia, but often ineffectively. Such legumes as *Centrosema* spp., *Desmodium*, some *Stylosanthes* spp., and *Phaseolus vulgaris* L. belong to this group. More recently, Date (1982) has proposed a similar specificity classification scheme for tropical forage legumes that include three categories: (i) Group PE, Promiscuous and Effective; (ii) Group PI, Promiscuous and Ineffective; and (iii) Group S, Specific.

Even among the promiscuous group, there are those that nodulate with a subgroup of bacteria. Earlier, Doku (1969) observed that all bacteria derived from nodules of peanut (*Arachis hypogaea* L.) and Bambarra groundnut [*Vigna subterranea* (L.)] were capable of nodulating each other as well as cowpea [*V. unguiculata* (L.)] and lima bean (*Phaseolus lunatus* L.). However, not all isolates nodulating cowpea were able to nodulate peanut and Bambarra groundnut. In addition, Ahmad et al. (1981) showed that not all of the West African isolates from cowpea, a promiscuous legume which is thought not to require rhizobial inoculation in tropical soils (Sellschop,

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1962), were capable of nodulating peanut, pigeonpea, or mungbean [*V. radiata* (L.)], which are all classified in the "cowpea miscellany" cross-inoculation group.

## THE MYTH OF THE LACK OF LEGUME RESPONSE TO INOCULATION IN THE TROPICS

### International Network of Legume Inoculation Trials

The myth that tropical legumes do not need inoculation, and the lack of hard evidence to the contrary, had created a void in the supply of, and demand for, inoculants in most developing countries. In 1979, Univ. of Hawaii's Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL) Project organized a planning workshop to develop an experimental protocol and strategy for implementation of standardized inoculation trials on a global scale (Harris, 1979). Twenty-nine agricultural, biological and social scientists, biometricians, administrators, and management specialists worked together to develop appropriate experimental designs and procedures to be implemented by volunteer cooperators in developing countries. The primary purpose of this 5-yr effort was to determine under realistic field conditions whether yield of agriculturally important tropical legumes could be enhanced by rhizobial inoculation. An added benefit was that of demonstrating the potential usefulness of BNF technology for agricultural development to farmers, extension workers, scientists, and decision makers. The scope of International Network of Legume Inoculation Trials (INLIT) encompassed a standard set of experiments to test inoculation response of legumes under two levels of fertility (farmers' local practices and maximal level), and in comparison with added fertilizer N. INLIT cooperators conducted more than 200 standardized experiments with 19 species of legume field sites in more than 20 countries.

The treatments, originally designed by the INLIT Workshop participants (Harris, 1979), were considered the minimum that needed to be done to address the objectives in a realistic and statistically reliable manner. Treatments for the INLIT trials included three N-source treatments: (i) inoculated; (ii) uninoculated; and (iii) fertilizer N. These N-source treatments were conducted at two levels of management: (i) farmer conditions; and (ii) maximal management (for details of design and analysis see Davis et al., 1985). All cooperators were urged to adhere to standardized guidelines for plot layout, dimensions, and planting information.

A cumulative summary of the data from INLIT is given in Table 8-2. In a large number of trials, a significant increase (>1.0 SD) was obtained under both fertility levels. Even legumes that belong to the "cowpea miscellany" cross-inoculation group (*A. hypogea, C. cajan, V. mungo, V. radiata*, and *V. unguiculata*) had significantly higher yields with rhizobial inoculation in a large percentage of the trials. In 29% of the cases, improving other fertility factors increased the chances of inoculation success. Without

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Table 8-2. Yield response of tropical legumes to rhizobial inoculation: Summary of results.†

| Scientific name | Common name | A Total trials | B % A, CV <20% | Significant response to inoculation, % of total‡ Fertility level Farm | Max. | Management |
|---|---|---|---|---|---|---|
| *Arachis hypogaea* L. | Peanut | 26 | 88 | 50 | 46 | 23 |
| *Cicer arietinum* L. | Chickpea | 31 | 61 | 48 | 55 | 23 |
| *Cajanus cajan* (L.) | Pigeonpea | 8 | 38 | 13 | 13 | 88 |
| *Glycine max* (L.) | Soybean | 40 | 62 | 65 | 65 | 30 |
| *Lens culinaris* Medikus | Lentil | 27 | 78 | 48 | 41 | 11 |
| *Leucaena leucocephala* (Lam.) | Leucaena | 8 | 63 | 38 | 50 | 25 |
| *Phaseolus vulgaris* L. | Common bean | 10 | 80 | 10 | 30 | 50 |
| *Vigna mungo* (L.) | Gram, black | 15 | 67 | 53 | 60 | 47 |
| *Vigna radiata* (L.) | Mung bean | 40 | 60 | 70 | 68 | 28 |
| *Vigna unguiculata* (L.) | Cowpea | 9 | 56 | 56 | 11 | 11 |
| Other§ | | 14 | 71 | 36 | 50 | 21 |
| Total | | 228 | | | | |

† Compiled from data of cooperators of the International Network of Legume Inoculation Trials (INLIT), sponsored by NifTAL Project, coordinated by R.J. Davis. Column A = No. of trials acceptable out of those analyzed; B = percent of A with CV <20%.

‡ Percent of column A, with yield of inoculated greater than uninoculated by greater than SD. Management refers to the percent of trials in which inoculated treatments at max. were significantly greater than those at farm fertility.

§ "Other" includes: *Aeschynomene americana* (1), *Calopogonium caeru* (1), *Centrosema pubescense* (1), *Medicago sativa* (1), *Pisum sativum* (5), *Psophocarpus tetragonolobus* (2), *Pueraris phaseoloides* (1), *Sesbania sesban* (1), and *Vicia faba* (1).

additional environmental information, it is difficult to explain why some cooperators obtained a positive inoculation response at farm fertility levels, but not at maximal fertility. Such are the vagaries of field trials conducted on different species by different individuals at different sites.

A breakdown of the INLIT results by country (Table 8-3) shows that legumes that respond to inoculation in one country do not necessarily do so in another. The pattern of response, however, does not correspond strictly to the center of origin and diversity of the legume species. This variability indicates that there is enough local heterogeneity and site specificity to account for a legume species responding to inoculation in its own center of origin. It should be noted that some of the trials were conducted at government and university experimental stations and, therefore, were affected by prior experimental agricultural practices that may or may not have included rhizobial inoculation.

## Measuring the Response of Legume Inoculation: Limitations of Standard Inoculation Trials

Recommending that farmers inoculate or that national programs and private industry invest resources to enhance the delivery of BNF technology is predicated on the results of inoculation trials. INLIT demonstrated that

Table 8-3. Distribution of yield response of specific legumes by country at two levels of fertil'.y.

| Legume species | Total no. | Response at fertility† Farm | Max. |
|---|---|---|---|
| *Arachis hypogaea* L | | | |
| Argentina | 1 | 1 | 0 |
| Burma | 1 | 0 | 0 |
| Ghana | 2 | 2 | 0 |
| India | 6 | 2 | 2 |
| Indonesia | 4 | 2 | 2 |
| Malaysia | 1 | 0 | 0 |
| Philippines | 9 | 5 | 6 |
| Rwanda | 1 | 0 | 1 |
| Vietnam | 1 | 1 | 1 |
| Total | 26 | 13 | 12 |
| *Cajanus cajan* (L.) | | | |
| Fiji | 3 | 1 | 0 |
| India | 5 | 0 | 1 |
| Total | 8 | 1 | 1 |
| *Cicer arietinum* L. | | | |
| Bangladesh | 3 | 3 | 3 |
| Burma | 1 | 0 | 0 |
| India | 22 | 10 | 11 |
| Nepal | 1 | 0 | 0 |
| Pakistan | 3 | 1 | 1 |
| USA | 1 | 1 | 0 |
| Total | 31 | 15 | 15 |
| *Glycine mas* (L.) | | | |
| Colombia | 1 | 0 | 0 |
| Egypt | 1 | 1 | 0 |
| Ethiopia | 2 | 0 | 1 |
| India | 2 | 1 | 1 |
| Indonesia | 4 | 2 | 1 |
| Malaysia | 2 | 1 | 2 |
| Mauritius | 1 | 1 | 1 |
| Nepal | 2 | 0 | 0 |
| Pakistan | 3 | 3 | 3 |
| Philippines | 9 | 7 | 6 |
| Rwanda | 1 | 1 | 1 |
| Sri Lanka | 1 | 0 | 0 |
| Sudan | 1 | 1 | 1 |
| Taiwan | 1 | 1 | 1 |
| Thailand | 1 | 1 | 1 |
| USA | 1 | 1 | 1 |
| Vietnam | 3 | 2 | 3 |
| Total | 36 | 23 | 23 |
| *Lens culinaris* Medikus | | | |
| Bangladesh | 1 | 0 | 1 |
| India | 22 | 11 | 10 |
| Nepal | 2 | 2 | 0 |
| Pakistan | 1 | 0 | 0 |
| USA | 1 | 0 | 0 |
| Total | 27 | 13 | 11 |
| *Leucaena leucocephala* (Lam.) | | | |
| Brazil | 1 | 1 | 1 |
| India | 2 | 0 | 0 |
| Mexico | 1 | 0 | 1 |
| Philippines | 2 | 2 | 1 |
| Puerto Rico | 1 | 0 | 1 |
| USA | 1 | 0 | 0 |
| Total | 8 | 3 | 4 |
| *Phaseolus vulgaris* L. | | | |
| Brazil | 1 | 0 | 0 |
| Cameroon | 1 | 0 | 0 |
| Ethiopia | 3 | 0 | 2 |
| Guatemala | 1 | 0 | 0 |
| India | 1 | 0 | 0 |
| Indonesia | 1 | 1 | 1 |
| Mexico | 1 | 0 | 0 |
| USA | 1 | 0 | 0 |
| Total | 10 | 1 | 3 |
| *Pisum sativum* L. | | | |
| Guatemala | 1 | 0 | 1 |
| India | 4 | 2 | 2 |
| Total | 5 | 2 | 3 |
| *Vigna Mango* (L.) | | | |
| Bangladesh | 1 | 0 | 0 |
| India | 14 | 8 | 9 |
| Total | 15 | 8 | 9 |
| *V. radiata* L. | | | |
| Bangladesh | 1 | 0 | 0 |
| Burma | 1 | 0 | 0 |
| Fiji | 1 | 0 | 0 |
| India | 23 | 17 | 17 |
| Pakistan | 3 | 2 | 2 |
| Philippines | 10 | 9 | 7 |
| Swaziland | 1 | 0 | 1 |
| Total | 40 | 28 | 27 |
| *V. unguiculata* (L.) | | | |
| Fiji | 2 | 0 | 0 |
| India | 6 | 2 | 1 |
| Rwanda | 1 | 0 | 0 |
| Total | 9 | 2 | 1 |

† Number of experiments with yield of inoculated greater than uninoculated by at least 1.0 SD. Results from USA are from INLIT trials conducted in Hawaii.

Table 8-4. Frequency of inoculation response and increase in seed protein content. (From J. Thies, Ph.D. thesis, Univ. of Hawaii, 1990.)

| Legume protein | Inoculation increased | | Average seed N | |
|---|---|---|---|---|
| | Yield | Seed N | + inoc. | − Inoc. |
| | % of trials | | g N $kg^{-1}$ | |
| Soybean | 83 | 100 | 38.8 | 35.6 |
| Lima bean | 60 | 80 | 19.4 | 18.8 |
| Common bean | 33 | 50 | 18.8 | 17.5 |
| Cowpea | 0 | 80 | 26.3 | 24.4 |

the yield response to legume inoculation is highly variable and can be difficult to measure statistically with small plot field experiments. INLIT results suggest that other criteria for measuring the response to legume inoculation and standards for accepting results should be considered.

### Limitations of Measuring Yield Response

The benefit to inoculation must be considered in the context of the small investment required to inoculate legumes. Table 8-4 shows that inoculation increased seed protein content more frequently than yield. While yield is a major criterion, better seed quality, total N increases, or conservation of soil N should also be considered as criteria for a positive result from inoculation trials.

It makes little sense that experimental results meet rigid statistical tests prior to recommending inoculation to the farmer. The variability inherent in even well-managed small plot field experiments is large. Only under the best experimental management are coefficients of variation (CV) $<15\%$ obtained. This variation precludes measuring statistically significant results of any single experiment when, depending on average yield, the yield increase is $<300$ kg $ha^{-1}$. Given the low cost of inoculant, measuring an economically relevant response to legume inoculation in terms of yield is limited when traditional statistical tests are applied to field experiments. New methods of measurement and predicting benefits from legume inoculation are clearly indicated if BNF researchers are to make valid recommendations on the need to develop and deliver inoculation technology to farmers.

### The Site-Specific Nature of Response to Inoculation Trials

Although field experimentation is a standard technique for measuring the response to inoculation, the manner in which inoculation trials have been performed in the past preclude obtaining results of relevance to locations other than the site where they were performed. Most trials were conducted without a measure of any factor that may affect or correlate with the response to inoculation. The results of these individuals trials and reasons for inoculation response remain site specific in both time and space. Using the results of these trials to project the performance of inoculant at other sites assumes that environmental, soil, and biological factors do not play a role in determining the response to legume inoculation.

While many trials were designed to determine legume response to inoculation under local conditions, others had regional or global perspectives. Programs such as INLIT (Harris, 1979), or those sponsored by IPB (Nutman, 1976) and International Center of Tropical Agriculture (CIAT), Cali, Columbia, were intended to demonstrate the need for inoculant with numerous crops in a variety of environments using standard experimental protocols. These programs were effective in demonstrating that some legumes responded to inoculation in some environments. Compared to single location trials, the results of these programs offer more information and can be used to generate a probability statement of expected response to inoculation by a legume over the range of conditions included in the trials. The usefulness of this information is, however, restricted since the soil and climate conditions of the trials are not specified sufficiently to identify particular situations where a response is likely. Neither planning agencies, industry, nor farmers and extension workers can confidently use this information as criteria to develop or promote inoculation technology under their specific conditions.

Understanding the factors that regulate the response to legume inoculation may lead to quantitative models that can predict the need for, and the benefit from, legume inoculation in any environment. Following is a discussion on factors regulating the response to inoculation and some experimental results that indicate a new approach for determining whether farmers are likely to benefit from inoculation.

## A New Approach to Inoculation Trials: The Worldwide Rhizobial Ecology Network

The Worldwide Rhizobial Ecology Network (WREN) was established with the hypothesis that measurable environmental factors determine and could be used to predict the performance of rhizobial inoculant in tropical soils. Similar to INLIT and other inoculation programs, the WREN developed a standardized set of experimental protocols to measure the response to inoculation. A network of collaborators was established in 17 countries in the tropics. Collaborators were selected on the basis of their experience with earlier networks and rhizobial technology.

WREN is unique in that the site specificity of inoculation trials was formally addressed. Experimental protocols included measures of factors most influencing the response to inoculation. Specific protocols included in the design of WREN experiments include: (i) a non-$N_2$-fixing legume species to evaluate the availability of soil N; (ii) a fertilizer N control providing N in excess of that required for growth to measure yield potential; and (iii) evaluation of several legume species with different rhizobial requirements at the same site. A database of indigenous rhizobial numbers and soil and environmental parameters was developed for each site.

While many parameters were measured, there were three which, when combined, explained more than 80% of the variation between results of the inoculation trials. These factors are: (i) yield potential; (ii) soil N availability; and (iii) the size of the indigenous population of rhizobia. The most im-

portant of the three factors is the number of rhizobia in the soil at planting. Mathematical models describing the relation between these factors and the response to inoculation have been reported (Thies et al., 1991a, b).

## Regulation of the Response to Legume Inoculation: A Conceptual Model

A conceptual framework of the factors influencing the response to legume inoculation is presented in Fig. 8-1. Legumes have two sources from which to assimilate N: (i) mineral N from the soil or fertilizer and (ii) the atmosphere. Total potential N assimilation (Crop Demand for Nitrogen) occurs in proportion to growth, and is determined by a complement of soil and environmental factors in the system. Mineral N availability above small quantities in early seedling growth reduces assimilation of atmospheric N (George et al., 1988; Herridge & Brockwell, 1988; Imsande, 1986; Ralston & Imsande, 1983) and regulates the crop N deficient that must be met through BNF. Therefore, the relationship between legume-N requirement and soil-N availability defines the potential amount of atmospheric N required by the legume.

The crop N deficit can be met through BNF only if there are sufficient quality and quantity of rhizobia available from the soil and inoculant sources. The potential crop response to inoculation is then defined by the quality of the indigenous rhizobia in the soil at inoculation. When populations of rhizobia in the soil are insufficient to meet the demand for fixed N by the legume, a response to inoculation will be observed. The important variables in this conceptual framework are addressed below. It is certain that unless there is a quantitative understanding of these regulating variables and their interaction, further inoculation trials will continue to yield the same site-specific information as the multitudes of earlier trials.

### Factors Affecting the Response to Inoculation

**Yield Potential of Legumes and the Response to Inoculation.** Environmental and management variables influence legume yield and, as a result, the requirement for atmospheric N. Nitrogen fixation and the potential response to inoculation, therefore, are also necessarily affected by the en-

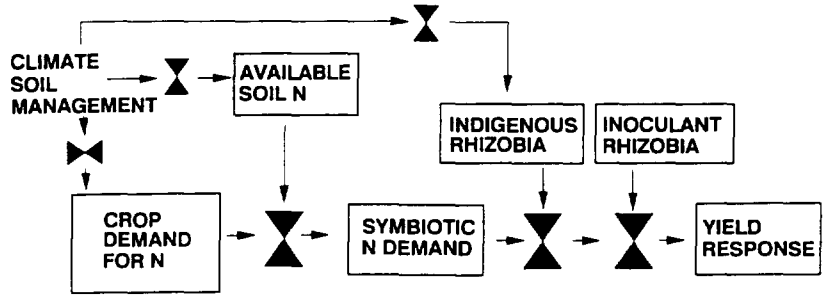


Fig. 8-1. Conceptual model of the regulation of the response to legume inoculation.

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vironment. Management and environmental considerations have received little attention in relation to $N_2$ fixation and the response to legume inoculation with rhizobia. Based on the conceptual framework presented in Fig. 8-1, the influence of management and environment on the potential response to legume inoculation is a function of their relative impacts on plant growth (crop N demand), rhizobia, and symbiotic processes (N supply).

Despite the many symbiotic processes that may influence the response to inoculation, most research to improve symbiotic performance in the field has focused on strain selection for effectiveness (Boonkerd et al., 1978; Ham et al., 1971; Harris, 1979) and environmental influences on rhizobia as free-living organisms (for a comprehensive review see Lowendorf, 1980). Significant effort has been directed toward selecting rhizobia for tolerance to environmental stress (Cassman et al., 1981a, b; Keyser & Munns, 1979) with the goal of improving symbiotic performance in environments with ecological constraints to legume productivity. While the saprophytic phase is an important determinant of the population size of indigenous rhizobia and survival of rhizobia in inoculant, it is often not limiting the response to legume inoculation. Rhizobia are generally more tolerant than the host plant to many soil stresses.

Figure 8-2 shows the result of an inoculation trial with soybean using two strains of *Bradyrhizobium japonicum* that had been shown to differ in ability to grow in cultures low in available P (Cassman et al., 1981a, b). Strain USDA 110 performed better in vitro under P-limited conditions than strain

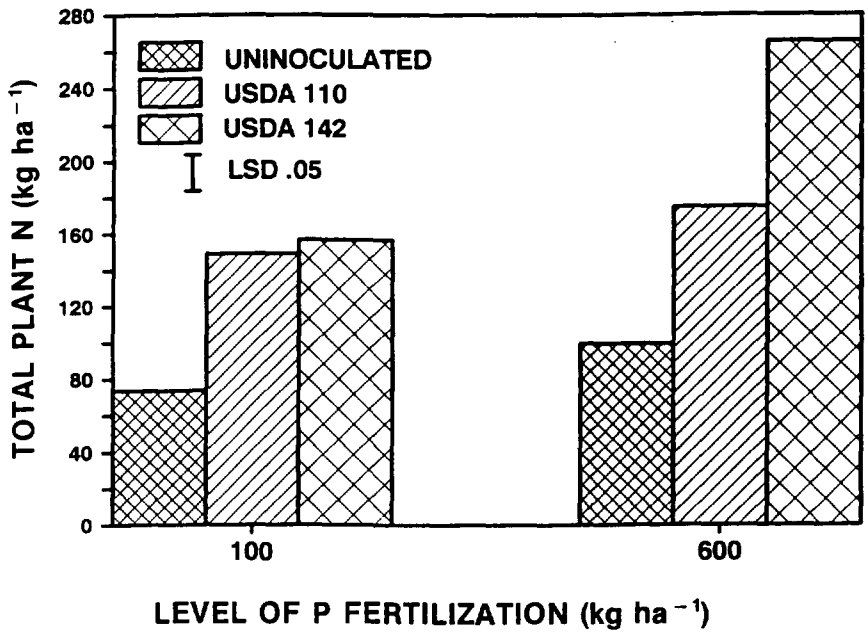


Fig. 8-2. Effect of P and strain of *Bradyrhizobium japonicum* on the response to inoculation of soybean.

USDA 142. Comparing the performance of the two strains in a P-deficient soil indicates that the P status of the soil regulated the response to inoculation (R. Nyemba, M.Sc. thesis, Univ. of Hawaii, 1986). Strain USDA 142 performed as well as USDA 110 under P-limited conditions but fixed significantly more N when additional P was added. Differences between strains were not large until the P constraint was completely removed, indicating that BNF effectiveness was fully expressed only when crop N demand was enhanced by P fertilization. Similar conclusions regarding P availability and rhizobial performance have been reached in controlled greenhouse experiments (Singleton et al., 1985).

There have been few reports that identify which stages of the symbiotic process are most sensitive to environmental stresses. The lack of research in this area is due to the difficulty in isolating the effect of environment on particular processes. Various approaches have been used to identify the first limiting process of the symbiosis in conditions of environmental stress. The approaches have either isolated the effects of environment on nodule formation or function in time (Munns, 1968), space (Hinson, 1975; Singleton & Bohlool, 1983, 1984), or by enhancing the supply of photosynthate supply in conditions of soil moisture deficit through enhanced $CO_2$ concentration (Huang et al., 1975).

Results from these limited number of studies indicate that soil stress factors such as drought (Huang, 1975) and salinity (Singleton & Bohlool, 1983) did not directly impact nodule function. The effects of these soil stress factors on an existing symbiosis was through a reduction in growth and photosynthesis. Nodule formation has been shown to be more sensitive to soil acidity (Munns, 1968) and salinity (Singleton & Bohlool, 1983) than plant growth of alfalfa and soybean. The presence of mineral N does not reduce plant growth and affects the infection process more than nodule function (Hinson, 1975). While limited, these reports indicate that under some agricultural environments, the magnitude of response to inoculation will be mainly influenced by general crop adaptation rather than specific direct impacts of environment on symbiotic processes.

**Soil Nitrogen Availability.** The difference between soil N available to the crop and crop N requirements determines the N deficit that must be met by BNF. While many agricultural soils cannot supply N in sufficient quantities to eliminate the need for BNF, the amount of N available can be a major factor contributing to the magnitude of the response to inoculation.

Many reports exist on the effects of mineral N on nodulation and $N_2$ fixation by legumes (see Munns, 1977, for review). It is generally accepted that small quantities of mineral N available during early growth can promote nodulation and $N_2$ fixation. This increase is most likely due to increased plant vigor during the establishment of symbiotic structures. Additional available mineral N substitutes for $N_2$ fixation, and if the mineral N is supplied in abundance, it can completely suppress the symbiosis (Gibson & Harper, 1985; Ismande, 1986).

Table 8-5. The effect of potential crop N deficit on soybean response to inoculation. (Data from George, 1988.)

| Crop N deficit† | Inoculation response |
|---|---|
| kg ha$^{-1}$ | % |
| 0 | 5.8 |
| 7 | 2.4 |
| 16 | 11.0 |
| 19 | 16.1 |
| 131 | 37.9 |
| 206 | 113.0 |

† Crop N deficit calculated by subtracting N assimilated by a nonnod soybean isoline from N assimilated by inoculated Clark soybean supplied with a total of 900 kg of N applied in three equal applications at planting, R2/R3, and R4/R5.

There are few reports that quantify the effect of mineral N on $N_2$ fixation and productivity in the field. Weber (1966) demonstrated that mineral N regulated the symbiosis of field-grown soybean over a 7-yr period. Nitrogen applied at rates up to 600 kg of N ha$^{-1}$ was required to completely suppress $N_2$ fixation. In years with reduced rainfall, $N_2$ fixation was eliminated with as little as 150 kg of N ha$^{-1}$.

Data in Table 8-5 demonstrates how the magnitude of crop N deficit affects the potenital response to inoculation of soybean. Crop N deficit for data in Table 8-5 (George, 1988) was calculated by subtracting the N assimilated by a nonnodulating soybean isoline from that assimilated by inoculated soybean supplied with 900 kg of N ha$^{-1}$. The lack of significant nodulation by the plus N control indicated that this treatment was measuring near-maximum N assimilation potential. The nonfixing soybean measured total soil N availability. The crop N deficit in effect integrates both soil N availability and yield potential in the system.

**Populations of Rhizobia.** Although the quality and size of indigenous populations of rhizobia regulates the response to inoculation, there are few reports where measures of rhizobial populations are correlated with crop performance. Indigenous populations of rhizobia are probably the single most important, but least studied, of any factor that conditions the response to legume inoculation.

The indigenous rhizobia in the soil present a barrier to increasing crop yield through inoculation. Native rhizobia compete with inoculant strains for nodulation of the host (Berg et al., 1988; Bohlool & Schmidt, 1973; Weaver & Frederick, 1974a, b). Indigenous populations of rhizobia also may be highly effective at $N_2$ fixation (Brockwell et al., 1988; Gibson et al., 1975; Singleton & Tavares, 1986). Following is a discussion of rhizobial populations in tropical soils, and their influence on the success of inoculum strains and response to legume inoculation.

Table 8-6. Frequency distribution of rhizobial population densities in tropical soils.†

| Number rhizobia‡ | Test host | | | | |
|---|---|---|---|---|---|
| | Soybean | Cowpea§ | Lima bean | Common bean | *Leucaena leucocephala* |
| $g^{-1}$ soil | % of samples | | | | |
| 0 | 47 | 16 | 26 | 35 | 51 |
| 1-10 | 13 | 9 | 26 | 11 | 9 |
| 10-100 | 15 | 21 | 21 | 19 | 15 |
| 100-1000 | 10 | 12 | 5 | 15 | 9 |
| >1000 | 14 | 40 | 21 | 19 | 15 |
| No. of samples | 105 | 122 | 19 | 26 | 33 |

† Data from WREN: Ecuador (G. Bernal; C. Estevez); Costa Rica (C. Ramirez); Philippines (E. Paterno); Egypt (H. Moawad); Honduras (J. Rosas); India (S.V. Hegde); Morocco (A. Hilali); Pakistan (K. Malik; F. Yusef); Ghana (R. Abaidoo); Puerto Rico (E. Schroeder); Taiwan (C.C. Young); Zimbabwe (M. Nyika); Zambia (R. Nyemba; V. Chinene); Brazil (W. Ribeiro); Thailand (N. Boonkerd; P. Wadisirisuk); Hawaii (Theis, Ph.D. thesis, Univ. of Hawaii, 1990; Woomer et al., 1988); Guyana (Trotman & Weaver, 1986); Indonesia (P. Singleton, B. Hilton, S. Saroso, and N. Boonkerd, 1990, unpublished data); and India (P. Singleton and S.V. Hegde, 1990, unpublished data).

‡ Zero indicates rhizobial numbers were less than the detection limit of 0.4 rhizobia $g^{-1}$ soil.

§ Hosts include: cowpea, mung bean, groundnut, and *Macroptilium atropurpureum* L.).

## THE MYTH OF THE UBIQUITY AND ABUNDANCE OF TROPICAL RHIZOBIA

Misconceptions about distribution of tropical rhizobia stem mostly from the confusion created by the taxonomic units to which these rhizobia are assigned, that is, cowpea miscellany, as discussed earlier. Data from 305 soil samples in the tropics (Table 8-6) illustrate that while rhizobia for cowpea and a few other promiscuous legumes are present in a large number of sites, the population densities are extremely variable. Almost one-half of the soils sampled had fewer than 100 rhizobia $g^{-1}$ soil. The distribution of rhizobia of legumes of the subgroups of the cowpea miscellany (e.g., *P. lunatus*) indicate that more soils have low numbers of rhizobia for these legumes. It simply cannot be assumed that tropical soils have sufficient numbers of *Bradyrhizobium* spp. to meet crop N demand.

Rhizobia for several of the important legumes grown in the tropics (i.e., *L. leucocephala* and *G. max*) are either not present at all in many of the locations, or occur in such low numbers that would necessitate inoculation.

### Ecological Factors Influencing the Indigenous Populations of Rhizobia

The presence and size of indigenous populations of rhizobia is a function of climate, soil, crop history, and management (Lawson et al., 1987; Weaver et al., 1987; Woomer et al., 1988; Yousef et al., 1987). Woomer et al. (1988) developed mathematical expressions describing the population density of six species of rhizobia in tropical soils in terms of climate, soil, and

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legume cover while Weaver et al. (1987) demonstrated the importance of crop history, and Yousef et al. (1987) described the importance of crop history and soil factors. Species of rhizobia were restricted to ecosystems where a homologous host grows. Rainfall, soil chemical properties, and the intensity of the legume component in the natural vegetation or crop system have been identified as the most important factors determining the size of rhizobial populations in soil (Woomer et al., 1988).

## Competition for Nodule Sites by Indigenous Rhizobia

Numerous reports indicate that competition from native strains of homologous rhizobia represent a constraint to establishing the inoculant on the root system and obtaining a response to legume inoculation. Strains differ in their competitive ability both in soil and artificial media (see Parker et al., 1977 for a review). Weaver and Frederick (1974a, b) and Bohlool and Schmidt (1973) concluded that success in establishing inoculant strains of rhizobia in nodules of soybean was a function of the population size in the soil. Others have concluded that competition is a major factor affecting the response to legume inoculation (Boonkerd et al., 1978; Johnson et al., 1965; Weaver & Frederick, 1974b).

A major criterion for selecting strains of rhizobia is that they will compete effectively with indigenous rhizobia to form nodules on the inoculated crop. The widespread distribution of *Bradyrhizobium* spp. in the tropics suggests competition is a major constraint to establishing inoculant strains in tropical soils. Little information is available on the conditions that affect competition between rhizobia in tropical soils. Table 8-7 demonstrates that inoculation increases the number of nodules on legumes growing in tropical soils. These data indicate that although competition is a problem in tropical soils, current inoculation technology can successfully establish superior strains of rhizobia on the root systems of tropical legumes even when there are significant numbers of indigenous rhizobia in the soil. It is doubtful that competition alone explains the failure of tropical legumes to respond to inoculation when quality inoculant and appropriate application methodology is practiced.

Table 8-7. Relative increase in nodule number from inoculation of legumes in soils with indigenous populations of rhizobia.†

| Indigenous rhizobia‡ | Nodule no. increase | No. observations |
|---|---|---|
| no. $g^{-1}$ soil | % | no. |
| 1-1000 | 434 | 12 |
| >1000 | 155 | 15 |

† Data from WREN: Egypt (H. Moawad); Ghana (R. Abaidoo); Thailand (N. Boonkerd; P. Wadisirisuk); Taiwan (C.C. Young); India (S.V. Hegde); Morocco (A. Hilali); Ecuador (C. Estevez; G. Bernal).

‡ Rates of inoculation range from $10^6$ to $10^7$ rhizobia per seed.

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## The Size of Indigenous Populations of Rhizobia: Impact on Response to Inoculation

The symbiotic potential of populations of rhizobia can be defined by their number and effectiveness at $N_2$ fixation with the host. The conceptual model indicates that if the crop demand for symbiotic N is met by the indigenous population, then there can be no increase in yield and $N_2$ fixation through inoculation. Brockwell (1977) concluded that although numerous surveys of naturally occurring rhizobia had been conducted, "investigations of occurrence, frequency, and effectiveness of rhizobia as a means of establishing or justifying the need for legume seed inoculation have been far less frequent." While passing reference to the presence of effective native rhizobia has been used to explain the failure of legumes to respond to inoculation (Diatloff & Langford, 1975; Ham et al., 1971; Meade et al., 1985), there are only a few reports that have attempted to measure the effect of indigenous rhizobia on the response to inoculation (Weaver & Frederick, 1974b; Singleton & Tavares, 1986; Thies et al., 1991a).

In a series of inoculation trials with soybean, Weaver and Frederick (1974b) did not observe a significant response to inoculation of soybean in six soils with indigenous *Bradyrhizobium japonicum* populations ranging from 11 to 229 086 rhizobia $g^{-1}$ of soil. Failure to respond to inoculation when there were even low numbers of rhizobia in the soil was attributed to soil N availability and competition from indigenous strains of rhizobia, even though the inoculant rhizobia formed more than 50% of the nodules when populations of indigenous rhizobia were below 10 000 per gram of soil.

Under more controlled experimental conditions, Singleton and Tavares (1986) isolated the effect of indigenous population size on the response to inoculation by eliminating soil N availability and competition from indigenous rhizobia as experimental variables. They did not measure significant increases in $N_2$ fixation by six legume species when indigenous homologous rhizobia were above 20 per gram of soil. Results of these experiments are presented in Fig. 8-3. Variation in relative response to inoculation when there are no indigenous rhizobia represents environmental impact on yield potential (in this case, the influence of time of year the experiment was conducted). Extensive field experimentation has recently confirmed this relationship between numbers of rhizobia in the soil and the response to inoculation (Thies et al., 1991a). From these experiments, quantitative models based on the number of indigenous rhizobia, soil N availability and the crop N deficit have been developed to describe the response to inoculation (Thies et al., 1991b). These models were developed with eight legume species including five grains, three pasture, and one tree legume.

It is surprising that such low population densities of rhizobia can support maximum $N_2$ fixation by legumes, and that $N_2$ fixation is so sensitive to small changes in numbers of rhizobia. The nonlinear relationship between the relative response to inoculation and population density of rhizobia in the soil at planting may be explained by three possibilities.

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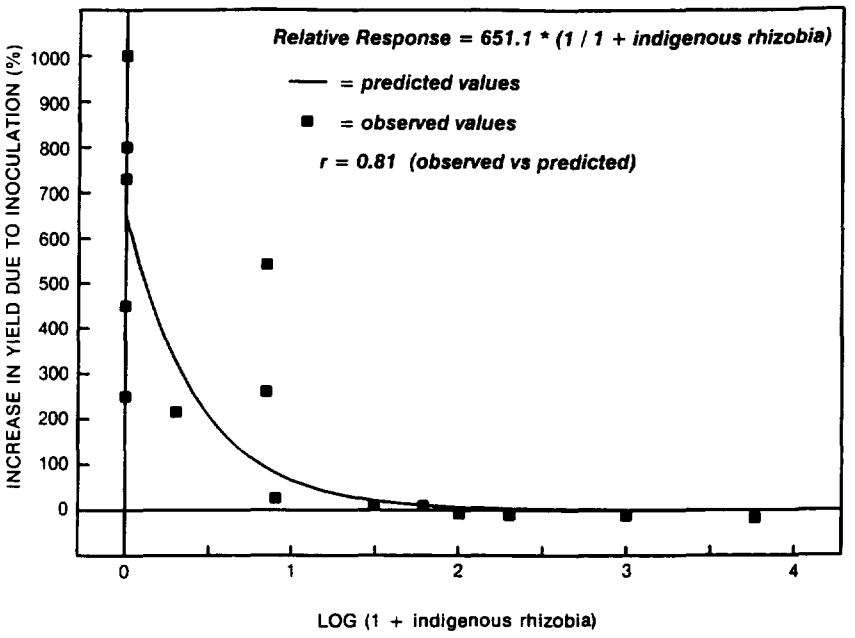


Fig. 8-3. Response to legume inoculation in relation to numbers of indigenous rhizobia: Greenhouse studies.

First, colonization of the roots and rhizosphere by rhizobia may play a role in increasing nodule number when there are few rhizobia in the soil. This mechanism has never been tested and is probably not significant. Otherwise, inoculation would not enhance nodule number as consistently as indicated in Table 8-7.

Second, even when population densities in the soil are low the root quickly exploits large volumes of soil, thereby increasing the total number of potential contacts with indigenous rhizobia. With populations of only 100 rhizobia $g^{-1}$ of soil, there are more rhizobia in 10 kg of soil than would be applied to a large-seeded legume through inoculation.

Third, the host compensates for low numbers of nodules formed on the plant when rhizobia in the soil are few in number by increasing the size of nodules (Singleton & Stockinger, 1983). The average size of soybean nodules can increase more than 200% when the number of effective nodules on the root system is reduced. The net result of this mechanism is that nodule tissue available to fix N is not a linear function of the number of nodules and the number of rhizobia in the soil. This mechanism may be a manifestation of legume adaptation to soil environments that vary in terms of the population of rhizobia available to support $N_2$ fixation.

Table 8-8 presents results from the WREN network and two additional sites in the Philippines. These experiments were carried out under high-management conditions to reduce the effects of environment between sites on legume response to inoculation. Results clearly indicate the effect that

Table 8-8. Influence of indigenous rhizobia on the response of legumes to inoculation with rhizobia tropical soils.†

| No. rhizobia | Experiments | Relative inoculation response‡ | Frequency of response | |
|---|---|---|---|---|
| | | | Inoculation | Nitrogen§ |
| no. $g^{-1}$ soil | no. | % | | |
| 0 | 14 | 252 | 71 | 86 |
| 1-10 | 16 | 94 | 92 | 71 |
| 1-100 | 13 | 14 | 70 | 70 |
| 100-1000 | 11 | 10 | 77 | 77 |
| >1000 | 14 | 8 | 71 | 85 |

† Data from WREN: Puerto Rico (E. Schroeder); Ecuador (G. Bernal; C. Estevez); Morocco (A. Hilali); Taiwan (C.C. Young); Thailand (N. Boonkerd; P. Wadisirisuk); Zambia (R. Nyemba; V. Chinene); Egypt (H. Moawad); Hawaii (J. Thies, Ph.D. thesis, Univ. of Hawaii, 1990); Philippines (C. Escano, H. Layaoen, Y. Castroverde, and P. Singleton, 1990, unpublished data.)

‡ Legumes tested include: *Glycine max; Arachis hypogaea; Vigna unquiculata; Vigna radiata; Vicia faba; Leucaena leucocephala; Medicago sativa; Phaseolus lunatus; Phaseolus vulgaris; Trifolium repens; Vicia sativa; Lathyrus tingeatus; Trifolium subterraneum.* Response indicates inoculation increased seed yield or total crop nitrogen.

§ Nitrogen applications averaged >800 kg of N/(ha crop). Application rates recommended in the network were 150 kg of N every 2 wk during crop growth.

indigenous rhizobia have on the response to inoculation. Results from inoculation trials in the field are similar to results of the model presented in Fig. 8-3 and those of Thies et al. (1991b). The response to inoculation is large when there are few or no rhizobia in the soil. The response declines rapidly when there are more than 10 rhizobia $g^{-1}$ of soil. The impact of indigenous rhizobia on the response to inoculation appears to be species neutral.

Results from six *Medicago sativa* experiments in the IBP inoculation trials measuring native rhizobia indicate an average relative increase in total crop N of 93% from inoculation when the indigenous rhizobial populations were <1 rhizobia $g^{-1}$ of soil (Nutman, 1976). There is insufficient data from IBP trials conducted in soils with larger indigenous populations of rhizobia to determine whether the relationship between population size and response to inoculation is similar to the tropical legumes presented in Table 8-7.

Quantitative models predicting the response to inoculation in terms of measurable soil and environmental parameters will have to incorporate some measure of population density as a primary driving variable. The impact of population density on the response to inoculation clearly indicates the need to increase the precision and ease with which rhizobia in soil can be measured.

## Effectiveness of Indigenous Rhizobia

Indigenous populations that are ineffective have been observed (Vincent, 1954; Jones et al., 1978). When populations of rhizobia are completely ineffective, a response to inoculation can be expected (Singleton & Tavares, 1986; Jones et al., 1978). Generally, however, populations of soil rhizobia are composed of numerous strains that vary in $N_2$-fixing capability with a

particular host (Bergerson, 1970; Gibson et al., 1975; Singleton & Tavares, 1986; Trotman & Weaver, 1986; Vincent, 1954). It is difficult to quantify the relationship between population effectiveness of indigenous rhizobia and the response to legume inoculation when the population contains strains with a range of symbiotic effectiveness.

Brockwell et al. (1988) developed a method to measure the $N_2$-fixing effectiveness of rhizobial populations. Test plants are inoculated with a variable amount of soil to provide a predetermined number of rhizobia in the inoculum. Indication of population effectiveness is determined by growth of the test plant. Another measure of population effectiveness tested the effectiveness of many random rhizobial isolates from the soil and developed frequency distributions of the effectiveness of isolates within the population (Singleton & Tavares, 1986; Trotman & Weaver, 1986). Both methods have limitations. The first method requires accurate enumeration of soil rhizobia to avoid confounding the effectiveness measurement with rhizobial numbers. The second method is limited by the amount of effort required to test population by single isolates. Unless the indigenous population of rhizobia is completely ineffective, measures of population effectiveness have not been good indicators of the magnitude of the response to inoculation.

## CONCLUSION

The myth that legumes do not respond to inoculation in the tropics is supported by general misconceptions about the promiscuity of tropical legumes and the ubiquity and abundance of tropical rhizobia. In reality, tropical legumes often exhibit some degree of specificity in their rhizobial requirements. In addition, many soils in the tropics do not contain sufficient indigenous rhizobial populations to meet the symbiotic potential of legume crops.

Limited data from previous site-specific inoculation trials have promoted the myth that tropical legumes do not respond to inoculation. While tropical legumes do not always respond to inoculation with sufficient magnitude to meet rigid statistical tests, the average response obtained from a range of tropical environments indicates that farmers will economically benefit from inoculation. Tropical legumes respond to inoculation in conditions fundamentally similar to those of temperate legumes. Specifically, the response to inoculation will be primarily controlled by the size and effectiveness of indigenous populations of rhizobia, and, to a lesser extent, by the yield potential of the crop and the availability of N from the soil.

Because the legume response to rhizobial inoculation involves a complex interaction between the crop, indigenous populations of rhizobia, and soil and environmental conditions, trials to determine the benefit from inoculation should account for these variables. These variables so profoundly affect the response to inoculation that site-specific results from inoculation trials are not reliable for making valid recommendations to farmers, government agencies, or private industry about the need to inoculate legumes.

The ultimate goal of continuing programs that involve inoculation trials should be to further quantify the complex interactions of variables determining the response to legume inoculation. Only then will it be possible to develop models based on soil and environmental test values that will predict the performance of legume inoculant in the field. Developing such capability is a prerequisite for determining where investment in inoculation technology is appropriate.

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## REFERENCES

Ahmad, M.H., A.R.J. Eaglesham, S. Hassauna, and B. Seaman. 1981. Examining the potential for inoculant use with cowpeas in West Africa. Trop. Agric. (Trinidad) 58:325-335.

Berg, R.K., R.M. Loynachan, R.M. Zablotowicz, and M.T. Lieberman. 1988. Nodule occupancy by introduced *Bradyrhizobium japonicum* in Iowa soils. Agron. J. 80:876-881.

Bergerson, F.J. 1970. Some Australian studies relating to the long term effects of the inoculation of legume seed. Plant Soil 32:727-736.

Bohlool, B.B., and E.L. Schmidt. 1973. Persistance and competition aspects of *Rhizobium japonicum* observed in soil by immunofluorescence microscopy. Soil Sci. Soc. Am. Proc. 37:561-564.

Boonkerd, N., D.F. Weber, and D.F. Bezdicek. 1978. Influence of *Rhizobium japonicum* strains and inoculation methods on soybeans grown in rhizobia-populated soil. Agron. J. 70:547-549.

Brockwell, J. 1977. Application of legume seed inoculant. p. 276-309. *In* R.W.F. Hardy and A.H. Gibson (ed.) A treatise on dinitrogen fixation. John Wiley and Sons, New York.

Brockwell, J., R.A. Holliday, and A. Pilka. 1988. Evaluation of the symbiotic nitrogen fixing potential of soils by direct microbial means. Plant Soil 108:163-170.

Burton, J.C. 1979. Rhizobium species. p. 29-57. *In* J. Peppler and D. Perlman (ed.) Microbial technology—Microbial processes. Academic Press, New York.

Cassman, K.G., D.N. Munns, and D.P. Beck. 1981a. Phosphorus nutrition of *Rhizobium japonicum*: Strain difference in phosphate storage and utilization. Soil Sci. Soc. Am. J. 45:517-520.

Cassman, K.G., D.N. Munns, and D.P. Beck. 1981b. Growth of *Rhizobium* strains at low concentrations of phosphate. Soil Sci. Soc. Am. J. 45:520-523.

Chen, W.X., G.H. Yan, and J.L. Li. 1988. Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int. J. Syst. Bacteriol. 38:392-397.

Date, R.A. 1982. Assessment of the rhizobial status of the soil. p. 85-94. *In* J.M. Vincent (ed.) Nitrogen fixation in legumes. Academic Press, Sydney, Australia.

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Davis, R.J., F.B. Cady, C.L. Wood, and C.P.Y. Chan. 1985. Design and analysis of an international experimental network: Legume inoculation trials in the NifTAL Project, the INLIT experience. HITAHR Res. Ser. 042. Coll. Trop. Agric. & Human Res., Univ. of Hawaii, Honolulu.

Diatloff, A., and S. Langford. 1975. Effective natural nodulation of peanuts in Queensland. Queens. J. Agric. Anim. Sci. 32:95-100.

Doku, E.V. 1969. Host specificity among five species in the cowpea cross-inoculation group. Plant Soil 30:126-128.

Dreyfus, B.L., J.L. Garcia, and M. Gillis. 1988. Characterization of *Azorhizobium caulinodans* gen. nov., a stem-nodulating, nitrogen fixing bacterium isolated from *Sesbania rostrata*. Int. J. Syst. Bacteriol. 38:89-98.

Evans, H.J., and L.E. Barber. 1977. Biological nitrogen fixation for food and fiber production. Science 197:332-339.

George, T. 1988. Growth and yield responses of *Glycine max* and *Phaseolus vulgaris* to mode of nitrogen nutrition and temperate changes with elevation. Ph.D. diss., Univ. of Hawaii, Honolulu.

George, T., P.W. Singleton, and B.B. Bohlool. 1988. Yield, soil nitrogen uptake, and nitrogen fixation by soybean from four maturity groups grown at three elevations. Agron. J. 80:563-567.

Gibson, A.H., B.L. Curnow, F.J. Bergerson, J. Brockwell, and A.C. Robinson. 1975. Studies of field populations of *Rhizobium*: Effectiveness of strains of *Rhizobium trifolii* associated with *Trifolium subterraneum* L. pastures in south-eastern Australia. Soil Biol. Biochem. 7:95-102.

Gibson, A.H., and J.E. Harper. 1985. Nitrate effect on nodulation of soybean by *Bradyrhizobium japonicum*. Crop Sci. 25:497-501.

Graham, P.H., and D.H. Hubbell. 1975. Legume-rhizobium relationships in tropical agriculture. p. 9-21. *In* E.C. Doll and G.O. Mott (ed.) Tropical forages in livestock production systems. ASA Spec. Publ. 24. ASA, Madison, WI.

Ham, G.E., V.B. Cardwell, and H.W. Johnson. 1971. Evaluation of *Rhizobium japonicum* inoculant in soils containing naturalized populations of rhizobia. Agron. J. 61:301-303.

Harris, S.C. 1979. Planning an international network of legume inoculation trails. NifTAL Project and Agency for Int. Develop., Paia, Hawaii.

Herridge, D.F., and J. Brockwell. 1988. Contributions of fixed nitrogen and soil nitrate to the nitrogen economy of irrigated soybean. Soil Biol. Biochem. 20:711-717.

Hinson, K. 1975. Nodulation responses from nitrogen applied to soybean half-root systems. Agron. J. 67:799-804.

Huang, C.Y., J.S. Boyer, and L.N. Vanderhoff. 1975. Limitation of acetylene reduction by photosynthesis in soybean having low water potentials. Plant Physiol. 56:228-232.

Imsande, J. 1986. Inhibition of nodule development in soybean by nitrate or reduced nitrogen. J. Exp. Bot. 37:348-355.

Johnson, H.W., U.M. Means, and C.R. Weber. 1965. Competition for nodule sites between strains of *Rhizobium japonicum*. Agron. J. 57:187-185.

Jones, M.B., J.C. Burton, and C.E. Vaughn. 1978. Role of inoculation in establishing subclover on California annual grasslands. Agron. J. 70:1081-1085.

Jordan, D.C. 1984. Family III Rhizobiaceae. CONN. 1938. p. 234-256. *In* N.R. Krieg (ed.) Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore.

Keyser, H.H., and D.N. Munns. 1979. Tolerance of rhizobia to acidity, aluminum and phosphate. Soil Sci. Soc. Am. J. 43:519-523.

Lawson, K.A., Y.M. Barnet, and C.A. McGilchrist. 1987. Environmental factors influencing numbers of *Rhizobium leguminosarum* biovar *trifolii* and its bacteriophage in field soils. Appl. Environ. Microbiol. 53:1125-1131.

Meade, J., P. Higgins, and F. O'Gara. 1985. Studies of inoculation and competitiveness of a *Rhizobium leguminosarum* strain in soils containing indigenous rhizobia. Appl. Environ. Microbiol. 49:899-903.

Munns, D.N. 1977. Mineral nutrition and the legume symbiosis. *In* R.W.F. Hardy and A.H. Gibson (ed.) A treatise on dinitrogen fixation. John Wiley and Sons, New York.

Munns, D. 1968. Nodulation of *Medicago sativa* in solution: Acid sensitive steps. Plant Soil 28:129-146.

Nutman, P.S. 1976. Field experiments on nitrogen fixation by nodulated legumes. *In* P.S. Nutman (ed.) Symbiotic nitrogen fixation in plants. Cambridge Univ. Press, Cambridge.

Nyemba, R.C. 1986. The effect of *Rhizobium* st.ain, phosphorus applied, and inoculation rate on nodulation and yield of soybean [*Glycine max* L.) Merr. cv. 'Davis']. M.Sc. Diss. Univ. of Hawaii, Honolulu.

Parker, C.A., M.J. Trinick, and D.L. Chatel. 1977. Rhizobia as soil and rhizosphere inhabitants. p. 311-352. *In* R.W.F. Hardy and A.H. Gibson (ed.) A treatise on dinitrogen fixation. John Wiley and Sons, New York.

Ralston, E.J., and J. Ismande. 1983. Nodulation of hydroponically grown soybean plants and inhibition of nodule development by nitrate. J. Exp. Bot. 34:1371-1378.

Scholla, M.H., and G.H. Elkan. 1984. *Rhizobium fredii* sp. nov., a fast-growing species that effectively nodulates soybeans. Int. J. Syst. Bacteriol. 34:484-486.

Sellschop, J.P.F. 1962. Cowpeas, *Vigna unguiculata* (L.) Walp. Field Crop Abstr. 15:259-266.

Singleton, P.W., and B.B. Bohlool. 1983. Effect of salinity on the functional components of soybean-*Rhizobium japonicum* symbiosis. Crop Sci. 23:815-818.

Singleton, P.W., and K.R. Stockinger. 1983. Compensation against ineffective nodulation in soybean. Crop Sci. 23:69-72.

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

Singleton, P.W., and J.W. Tavares. 1986. Inoculation response of legumes in relation to the number and effectiveness of indigenous *Rhizobium* populations. Appl. Environ. Microbiol. 51:1013-1018.

Thies, J.E., P.W. Singleton, and B.B. Bohlool. 1991a. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Appl. Environ. Microbiol. 57:19-28.

Thies, J.E., P.W. Singleton, and B.B. Bohlool. 1991b. Modeling symbiotic performance of introduced rhizobia in the field-based indices of indigenous population size and nitrogen status of the soil. Appl. Environ. Microbiol. 57:29-37.

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

Vincent, J.M. 1954. The root nodule bacteria of pasture legumes. Proc. Linn. Soc. N.S.W. 79:1-32.

Vincent, J.M. 1982. Role, needs, and potential of nodulated legumes. p. 263-284. *In* J.M. Vincent (ed.) Nitrogen fixation in legumes. Academic Press, Sydney, Australia.

Weaver, R.W., and L.R. Frederick. 1974a. Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. I. Greenhouse studies. Agron. J. 66:229-232.

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

Weaver, R.W., D.R. Morris, N. Boonkerd, and J. Sij. 1987. Populations of *Bradyrhizobium japonicum* in fields cropped with soybean-rice rotations. Soil Sci. Soc. Am. J. 51:90-92.

Weber, C.R. 1966. Nodulating and non-nodulating soybean isolines: II. Response to applied nitrogen and modified soil conditions. Agron. J. 58:46-49.

Woomer, P., P.W. Singleton, and B.B. Bohlool. 1988. Ecological indicators of native rhizobia in tropical soils. Appl. Environ. Microbiol. 54:1112-1116.

Yousef, A.N., A.S. Al-Nassiri, S.K. Al-Azawi, and N. Abdul-Hussain. 1987. Abundance of peanut rhizobia as affected by environmental conditions in Iraq. Soil Biol. Biochem. 19:319-396.