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SOME GUIDELINES FOR THE EVALUATION OF THE NEED FOR AND RESPONSE TO INOCULATION OF TROPICAL LEGUMES

by
Craig A. Meisner and H. Douglass Gross
Department of Crop Science
North Carolina State University

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SUMMARY OF GAPS IN KNOWLEDGE ABOUT INOCULATION OF TROPICAL AGRICULTURAL LEGUMES

There is need for:

1. National networks of tests for infective and effective strains of rhizobia that provide crop- and site-specific information;
2. appropriate long-term or rotation studies designed to provide information on nitrogen carry-over, rhizobium survival in the soil, and economic information on return to investment in inoculum;
3. comparisons of inocula with levels of nitrogen in order to demonstrate the degree to which biological fixation can substitute for commercial nitrogen fertilizer;
4. better communication between plant breeders and scientists responsible for rhizobial work to assure that host-strain interactions are considered as varieties are developed;
5. research on the possibility that pelleting with appropriate materials may substitute in part for broadcast fertilizer applications under peasant-farming conditions; and
6. research on the banding and placement of fertilizer as it may affect rhizobial survival and nodulation.

SYNOPSIS

Leguminous plants can derive nitrogen from the symbiotic (mutually-beneficial) relationship they form with Rhizobium bacteria. Inoculation involves the placement of the proper strain of rhizobium in the soil so that symbiosis may result. Benefits obtained from inoculation include: substantial saving in cost of nitrogen fertilizer, possible increase in soil nitrogen, increased yield of legumes that are nutritionally more adequate, and improved livestock production through better forage growth.

Many factors including soil temperature, pH, native fertility, and moisture as well as the competitive ability, infectiveness and nitrogen-fixing effectiveness of the rhizobial strains can influence the success of inoculation.

Because most legumes can provide enough nitrogen for their physiological needs the use of supplemental nitrogen has stimulated little or no yield increase and may be detrimental to the symbiotic relationship. Therefore, the use of nitrogen fertilizer, as compared to inoculation, is justified only in those few cases, such as with Phaseolus vulgaris, wherein inoculation has proven to be difficult or ineffective or those situations where an insuperable nutrient deficiency or climatic factor limits the N-fixation process.

There are many ways to inoculate legumes, each having its own advantages and disadvantages. These methods include simple slurries, using stickers or adhesives, and pelleting with amendments like lime or rock phosphate.

The response to inoculation is both site- and crop-specific. Therefore, recommendations cannot be broad-based in scope, particularly where inoculum availability is limited or costs are high. Simple field tests are presented that provide reasonable predicitive indices at both farmer's field and regional levels.

Under many circumstances the greatest advantage to legume inoculation may be not a yield increase *per se* but rather the elimination of the need for commercial fertilizer.

INTRODUCTION

Legumes* are widely distributed over the world as food, oil, and forage crops. They also exist as components of many native flora and, as such, often serve as range or browse species. Of the more than 17,000 species known, only about 200 are cultivated by man. These include the grain (pulse) types such as *Arachis hypogaea* (groundnut, peanut), *Cajanus cajan* (pigeonpea, red gram, arhar), *Cicer arietinum* (gram, chickpea, garbanzo, dhal), *Glycine max* (soybean), *Phaseolus spp* (the beans), *Pisum spp* (peas), *Vicia faba* (broadbean), *Vigna unguiculata* (cowpeas), and *V. radiata* (mung) that supply energy, protein and oil to much of the world's diet. Grain legumes supply up to 20% of the world's dietary protein needs; only about twelve species are commonly used in the tropics. Among the forage species are *Desmodium spp* (tick clovers), *Trifolium spp* (true clovers), *Medicago spp* (annual medics), *Centrosema spp*, and *Stylosanthes spp*. A few tropical food and feed legumes are listed in the Appendix.

Legumes are popular in agriculture because they have a reputation for enriching the soil, requiring little fertilizer Nitrogen (N), and yielding grain or forage high in protein which enhances the diet. This reputation, in large part, is valid and is due to a singular characteristic of the members of this plant family. In addition to the purely botanical criteria that distinguish the legumes, most of them are capable of being host to a bacterium, a member of the genus *Rhizobium*, that is able to use N from the air to satisfy its require-

*Strictly speaking the word "legume" refers to a type of fruit. In this paper it will be substituted for the more correct "leguminous plant."

ments for this nutrient element. This union, higher plant and the bacterium, that works to the mutual benefit of each is called a symbiotic relationship. In a sense the plant, the legume, is infected by the bacterium, the rhizobium. However, the plant gains N from the infection and the bacterium uses energy, fixed by photosynthesis, from the plant. Therefore, each benefits from the association.

This phenomenon, the symbiosis, is extremely important because the action of the bacterium involved is somewhat unique. Plants used in agriculture usually have a fairly high N requirement. Nitrogen is one of the elements essential to the synthesis of amino acids which, in turn, are used by the plant to form protein. If N is in low supply plant yield will usually be markedly reduced because proteins, necessary for cell production in both plants and animals, are not formed. The air that surrounds the earth is almost 80% N; this represents about 6400 kg N over each hectare. One would think that it would never be in short supply. But green plants cannot use this gaseous nitrogen. Their demands are met primarily by N in the ionic form of either ammonium (NH_4^+) or nitrate (NO_3^-). The ability of the rhizobial bacterium to take atmospheric N and convert it into a form that is usable by its host plant, the legume, is what makes this symbiosis so important to the farmer. In a very real sense he is getting his N fertilizer at no cost; the bacteria work gratis. The value of this "free" fertilizer N can be placed in global perspective if one considers that an estimated 50 million tons of N are fixed (manufactured) industrially each year; against this, an estimated 90 million tons are fixed by plant processes (35 million by crop legumes, 45 million in

permanent meadows and grasslands, and about 9 million tons by non-leguminous plants). Though the cost of a kilo of N may vary widely among regions, and may fluctuate wildly on the world market, it is obvious that the value of symbiotically-fixed atmospheric N is not inconsiderable. Of course there is a cost; it is represented by the energy supplied by the host plant to the bacteria in order that they may live and fix nitrogen. It has been estimated by plant biochemists that the symbiotic fixation process is about twice as energy-efficient as the commercial processes now used, all costs being considered. Thus, even if one estimated cost in terms of reduced yield, which is not straightforward, the legume-Rhizobium association, the symbiosis, would still be attractive. Though the amount of N fixed by legumes varies with crop and environmental conditions quantities up to 500 kg N/ha/yr have been recorded. The average leguminous crop probably fixes between 50 and 100 kg N/ha/yr.

Legumes may be grown for many purposes and the farmer may not be aware of the symbiotic relationship. In view of the fact that symbiosis does exist and that it represents an added benefit to the farming system, it certainly behooves professional agriculturalists to increase the farmers' awareness of the benefits and to increase symbiotic efficiency to the maximum. Nitrogen is an expensive fertilizer; expensive in terms of energy required to manufacture it and, usually, in terms of out-of-pocket cost. Moreover, in many parts of the world, because of communication and transport difficulties, it is virtually unavailable at any cost. Therefore, a knowledge of the value of symbiotic N-fixation is essential at another level. Agriculture-policy

makers should have some appreciation of the potential of the process. Decisions about importation of appropriate seed, the need for adequate inoculum-supply systems, or the merits of a domestic inoculum manufacturing and distribution network can be made best by informed administrators. It is in the best interest of agriculture worldwide, but particularly in developing countries with struggling agricultural economies, to take full advantage of the phenomenon we call symbiotic nitrogen fixation.

This paper seeks to summarize the present knowledge of the need for and advantages of symbiotic fixation under tropical conditions, to set forth some of the conditions that limit the efficiency or efficacy of the process, and to pinpoint some of the gaps in our knowledge that need further research.

LEGUME INOCULATION

The requirements for a successful symbiosis are definable and outwardly simple. They are: a reasonably healthy host-legume, capable of synthesizing enough carbohydrate to satisfy its own and the bacterial energy requirement; a strain (race) of *Rhizobium* compatible with the host, effective in assimilating atmospheric nitrogen; and an environment conducive to the vigorous growth of both organisms.

Anyone familiar with crop production at the field level will immediately grasp the fallacies inherent in assuming that the requirements described are easy to achieve. The basic step of producing a stout host-legume presents a myriad of problems at the small-holder level. Average yields of grain legumes in tropical environments tend to be rather modest; under peasant-farming conditions and in the develop-

ing countries they are very low indeed. It is not the purpose of this paper to discuss the constraints, apart from those affecting symbiosis, on grain or forage legume production in the tropics. Suffice it to say that any environmental factor that adversely impacts the growth of the host must have a depressing effect on the symbiotic process too.

Bacteria of the genus Rhizobium are fairly widespread in nature. However, there are several species within the genus, and many strains have been identified within some of the species. Data from temperate zone studies and, more recently, some that have involved tropical species indicate that some strains of Rhizobium tend to be rather host-specific. That is to say, a given species or, even, cultivar of crop legume may be effectively infected and may carry on an efficient symbiosis only with a particular strain of one species of Rhizobium. Thus, the second requirement set forth above is not necessarily easily fulfilled.

The final requirement mentioned was a favorable environment. Though under many circumstances rhizobia are remarkably durable and tolerant of what, in crop-production terms, would be most rigorous conditions, they can also be extremely sensitive to conditions in the field that must, of necessity, be considered if biological N fixation is to be maximized.

Other papers in this series discuss the matters of rhizobial strain selection, survival of the organisms in the soil, the production of suitable inocula, and so forth (see Section A of Bibliography). It is the purpose of this essay to discuss the value of the symbiosis and methods of achieving it. The method is called inoculation.

Legume inoculation is a way of assuring that the strain of rhizobium appropriate for the cultivar being seeded is present in the soil at the proper time and in numbers sufficient to assure a quick and effective infection and an efficient subsequent N fixation.

Inoculation is usually accomplished by treating the seed or the soil with a mix of bacteria and carrier, called inoculum. The more common commercial inocula in use today are mixtures of peat, or some other inert carrier, and a selected strain, or strains, of bacteria. These are mixed with moist, not wet, seed in such a manner that each seed is visibly coated with the carrier particles. Often an adhesive material, a sticker, is added to assure that the particles adhere to the seed coat; this sticker may also be a source of carbohydrate for the early growth of the bacteria. Milk, weak sugar or syrup solutions, or commercial stickers can be used. (Granular inocula, for direct application to the soil at seeding time, have recently come on the market. Their availability for crops other than soybeans is limited.)

After coating the seed with the inoculum, care must be taken that the treated seed are properly handled. Sunlight, in particular, because of the sterilizing effect of the ultraviolet rays, is lethal to the rhizobia. Some fertilizer, insecticide or herbicide materials are also very damaging. Treated seed should be carefully shaded and kept in a clean, dry place until it is sown. Normally it is best to sow inoculated seed on the day it is treated. If the inoculation has been done properly, and the seed sown promptly, the bacteria will begin to multiply shortly after they are placed in the soil. As the seed germinates and grows, the bacteria will penetrate (infect) the roots; the manner

of infection is not entirely clear but is seems to follow a pattern similar to that of pathogenic organisms. The result of this infection will be a growth, similar to a gall or the knots caused by some nematodes, that is called a nodule. A legume plant that has been properly inoculated and infected is referred to as "well nodulated." These nodules contain the bacteria, somewhat transformed physically, that carry out the assimilation of the nitrogen in the soil atmosphere. For most species, healthy nodules, if cut open, are bright red or pink inside. This is due to the presence of a compound called leghaemoglobin that, like the haemoglobin in our blood, regulates the supply of oxygen to the bacteria. This is one clue to effective N fixation. After from eight to twenty days, depending on the crop sown and the environment, bacteria, and nodules, should be present in sufficient number and activity high enough to supply the plant with its N needs. Under some field conditions this results in a spectacular "greening-up," comparable to that seen after a dressing of fertilizer N to a chlorotic crop.

EVALUATING THE NEED FOR INOCULATION

The need for inoculation can be evaluated at several levels; the yield of the crop that is not well nodulated, the quantitative and qualitative response of a crop to proper inoculation, the decreased cost (increased income) at the farm level, quantitative and qualitative increases in the products that reach the market, potential savings by the central government in subsidies for fertilizer import and distribution, and increased nutritional well-being of the population.

It is not within the scope of this paper to discuss the macroeco-

nomic, political or social benefits that accrue to an increased production of a quality product. Rather, the focus will be on the need at the field level. Qualitative and quantitative increases at the point of production can be extrapolated as one sees fit.

The presence of rhizobia in the field can be detected rather easily. One need merely look at the roots of legumes growing at the site. Almost all legumes, native or introduced, will show nodules on their roots. The presence of nodules is at least prima facie evidence of the presence of the bacteria. However, one should look more closely at the nodules, split them open and check the condition of the interior. The tissue should be sound, and the color pink or red. Only rarely are nodules effective if they do not show this color. White, green or black nodules usually indicate infection but not effective nitrogen-fixation. Nodules with a green base and red tissue above (abaxial or distal) indicate a previously functional relationship; the age and history of the crop should be checked.

The position and size of the nodules can also be an indication of the presence, number and effectiveness of the bacteria. Conventional wisdom has it that many large nodules on the main root, close to the crown (the juncture of root and stem), indicate abundant, competitive bacteria. Conversely, small nodules scattered widely on the secondary or finer roots are taken to indicate late nodulation, often delayed because of low bacterial populations, that is not very effective. These norms should not be interpreted too rigidly; there are many significant exceptions (C-3)*. However, they can certainly serve as guidelines.

*Refers to Bibliography listed at end of paper.

The physical appearance of the host plant is also a good indicator of the presence of effective bacteria. Most tropical soils are not high in nitrogen. Except for the well-known "spring flush," plants usually display N deficiency symptoms early in the growth cycle. A healthy, green legume is normally associated with adequate nodulation by effective bacteria. Chlorotic (yellow), stunted plants can result from many causes; moisture stress, disease, insect damage, nematodes, etc. But an unthrifty legume should always be examined for nodulation. Often farmers seek complicated or subtle explanations for poor legume crops without considering the possibility that the main limiting factor is inadequate nodulation caused by the lack of the proper rhizobial strain or low numbers of rhizobia.

The history of both the field and the crop should always be considered. If a natural stand is being evaluated, the appearance of associated species, particularly grasses, will often provide an indication of the general environmental impact on that season's growth. The grasses serve as indicators of the native nitrogen-supplying power of the soil. If patches of grass not close to the legumes are a healthy green the native N level must be considered adequate. Therefore, the condition of the legumes, nodulated or not, must be attributed in part to this basal N supply. If the grasses are not thrifty in appearance it may or may not be due to inadequate N supply, other factors such as nematodes may be operative.

In this case the legumes and their roots should be examined. If the legume plant is robust and green and the excavated roots are

nodulated, one may assume an adequate population of efficient rhizobia. If the legumes are pale green and not vigorous and the roots are well-nodulated the possibilities are that (1) the rhizobia that infected the root are ineffective or (2) that some other environmental factor is limiting growth. In either case further investigation is necessary.

If the legumes are not thrifty and the roots are not nodulated the conclusion is that no infective rhizobium is native to the soil in numbers adequate or at a time appropriate to cause nodulation. Inoculation in this case should increase plant growth unless a factor such as pH is limiting either the crop, the bacteria, or both.

Often legumes are seeded, or may be found, adjacent to fields that have received some fertilizer nitrogen. If legume growth is obviously improved in areas that have received drainage or runoff from the fertilized field one may conclude that the legume was suffering from inadequate or ineffective nodulation. Such a conclusion may not be drawn if the outflow is from a manure pile; other major or minor elements may be involved in this case.

The field history is important in that N-fertilized crop grown during the previous season may not have depleted the soil of nitrogen. Some residual effect should be considered. If, on the other hand, unfertilized grain or grass or the same legume has been grown on the field for several seasons, a thrifty crop would indicate adequate inoculation. An unsatisfactory crop might be due to inadequate or ineffective rhizobial populations but could also be caused by the build-up of pathogens, nematodes or destructive insect populations.

Another aspect of the crop history that must be considered is the

weather pattern preceeding observation. Drought, at that specific site, can cause the senescence of the nodules of many kinds of rhizobia. Legumes dug after such a drying cycle may bear no active nodules at all. However, some species of rhizobia can bring about a secondary infection of the host, often resulting in many small nodules on the finer plant roots. This kind of reinfection may be very effective in supplying the N needs of the host later in the season.

If a stand of a newly introduced legume is being evaluated, a good stand of healthy plants with robust, functional nodules is positive evidence of an adequate rhizobial population, native or introduced. In this instance the value of inoculation, relative to the efficacy of native rhizobia, can be determined only by testing, as discussed later.

It is often helpful, when a new legume, or inoculum, is being introduced into a district, to suggest to the farmer that he seed a few strips with uninoculated seed. The visual check provided by these strips pays excellent dividends for both the farmer and the field technician in evaluating the value of the new inoculum and the potency of the native population of rhizobia.

If few or no effective nodules are found on the legumes, native or introduced, a response to inoculation can certainly be anticipated and the objectives of any work planned for the future should be reasonably clear-cut, as discussed later. If, however, some effective nodules are found or if a native population is suspected of having caused the nodulation, a different set of questions needs to be posed:

1. What is the yield of economic product? Is the nodulation sufficient to support only a modest yield? Is natural nodulation adequate for economic crop production?
2. What is the cost of doing better? Will the probability of success increase if inoculation or a higher rate of inoculation is tried?
3. What would be the true cost if fertilizer N were used to achieve higher yields?
4. Are environmental factors other than N supply limiting yield?

These kinds of questions require more refined and objective testing.

FACTORS AFFECTING INOCULATION, NODULATION AND NITROGEN FIXATION

Factors influencing the symbiotic relationship may affect either of the organisms directly or the relationship between them; i.e., the plant may be affected or may cause an effect, the rhizobium may be affected or be the cause, the interaction or relationship between the two may be the cause and, finally, several environmental factors may impact the process in any of the above situations.

Most of the research on symbiotic N fixation has been conducted on temperate species and the rhizobia associated with them. Though such work has been going on since the turn of the century relatively few categorical conclusions may be drawn. Even less is known about the phenomenon under tropical conditions or on tropical species. Such research is only now beginning to be published with regularity.

The host plant may affect the symbiosis in several ways. Host plant management is discussed in other papers of this series. The matter

of the vigor of the host has already been mentioned. If the growth of the legume is adversely affected by any factor other than N deficiency the rhizobia will most likely also be affected. This is particularly true if any constraint is placed on either the photosynthetic or the translocation processes. Because the bacteria (bacteroids) in the nodules are totally dependent on the plant for energy and for carbohydrate skeletons with which to synthesize amino acids, and are also highly dependent on the plant's root system for their water needs, reduced photosynthesis, impeded translocation or curtailment of water absorption by the roots will tend to have immediate impact on the fixation process. Under tropical conditions, disease, root pruning (insects), soil compaction, poor drainage, and nutrient deficiences must be considered when evaluating the effectiveness of symbiotic fixation.

The host can also affect the nodulation process simply by being incompatible with the strain or species of Rhizobium that is attempting to infect it. The exact mechanism of this incompatibility is unknown, but so-called strain specificity has been demonstrated many times. For some plants, such as soybeans, the specificity may be identified genetically or can be defined at the plant variety level; for others it can be related to plant type and host carbohydrate metabolism, for example the bush-type vs. the climbers among field beans (Phaseolus vulgaris)[1/].

Finally, some plants, not usually hosts, can have a direct toxic effect on the microorganisms. This is one manifestation of the phenomenon called allelopathy; i.e., the toxic effect of one plant species on

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[1/] Dr. Jake Halliday, NifTAL, pers. comm.

another. It is usually associated with the leaf leachates or exudates from live plants, or the decomposition products from dead plants and residues. Its occurence is difficult to demonstrate; obviously the effects of competition would be very similar. A comparable problem is the demonstrated toxic principle in some legume seed coats that kills rhizobia. Pelleting (see below) may be of value if such toxicity is suspected.

Inoculation and nodulation problems attributed to the rhizobial symbiont may be due to presence or absence of appropriate strains, population numbers per se, low infectivity or effectiveness, competition among races of rhizobia, or poor survival in the soil.

Classically, species of the genus Rhizobium have been classified according to the leguminous hosts which they were able to nodulate. Such a scheme is shown in Table 1. Most of this type of work has been done with temperate legumes; in large part, the tropical legumes fall under the nebulous category of "cowpea miscellany."

An "agronomic" listing of crop species common to the groups shown in Table 1 is presented in Appendix Table II. This listing, though a useful guide, does not represent the final word in strain specificity, particularly with respect to tropical cultivars.

A third approach to rhizobium classification was suggested by Date (C-1). It is presented in Table 2 and is based on both field and laboratory observation. It compares to the microbiological breakdown suggested by Graham (D-4) and shown in Table 3.

Though the traditional scheme is no longer regarded as absoulte, no widely accepted alternative, particularly for tropical species, has

Table 1. Species of Rhizobium and their characteristics. (After Breed et al., 1957)

Species	Litmus milk		Growth Rate	Host nodulated	Common name of group
	Serum Zone	Acid Reaction			
R. trifolii	+	-	Fast	Trifolium	Clover
R. leguminosarum	+	-	Fast	Pisum, Lens, Lathyrus, Vicia	Pea
R. phaseoli	+	-	Fast	Phaseolus vulgaris	French bean
R. meliloti	+	+	Fast	Melilotus, Medicago, Trigonella	Alfalfa
R. japonicum	-	-	Slow	Glycine max	Soybean
R. lupini	-	-	Slow	Lupinus, Ornithopus	Lupin
Rhizobium spp.	Variable	Variable	Variable	Vigna, Desmodium, Arachis, Centrosema, Stylosanthes, etc.	Cowpea miscellany

From: Graham, D-4

Table 2. Inoculation groups for tropical pasture legumes based on effectiveness of nitrogen fixation with a range of strains of Rhizobium.

GROUPE PE — Nodulate effectively with a wide range of strains (=Old Cowpea-type group). Genera listed forming one loose group.

Arachis	Lablab	Psophocarpus
Calopogonium	Macroptilium	
Cajanus	Macrotyloma	
Canavalia	Pueraria	
Clitoria	Rhynchosia	
Crotalaria	Stizolobium	
Cyamopsis	Stylosanthes (several sub-groups)	
Desmanthus	Teramnus	
Dolichos	Tephrosia	
Galactia	Vigna	
Glycine	Zornia	
Indigofera	Gliricidia	

GROUP PI — Nodulate with a range of strains but often ineffectively. Genera listed forming individual groups with some crossing between groups. Sub-groups distinguishable.

Centrosema (2 sub-groups)	Adesmia
Desmodium (2 sub-groups)	Aeschynomene
Stylosanthes	Psoralea
	Sesbania (2 sub-groups)

GROUP S — Nodulate effectively with specific strains only. Genera listed forming specific groups.

Cicer	Coronilla
Leucaena	Lotus) 2 sub-groups
Lotononis - Listia (minimum 3 sub groups)	Lupin)
Medicago - Trigonella	
Mimosa	Lupinus
Stylosanthes (2 sub-groups)	
Trifolium (African) (4 sub-groups)	

Taken from R. A. Date, C-1.

Table 3. Species of Rhizobium and their characteristics. (After de Ley, 1968)

Species	Relation to species of Breed et al. (1957)	Flagellation	Per cent G+C	Litmus milk Serum Zone	Litmus milk Acid reaction	Growth Rate	Nodule forming characteristics, special features
R. leguminosarum	R. phaseoli + R. trifolii + R. leguminosarum	Peritrichous	59.0-63.5	+	-	Fast	Forms nodules on one or more of Trifolium, P. vulgaris, Vicia, Pisum, Lathyrus, Lens
R. meliloti	Unchanged	Peritrichous	62.0-63.5	+	+	Fast	Forms nodules with Melilotus, Medicago, Trigonella
R. rhizogenes	A. rhizogenes	Peritrichous	61.0-63.0	+	-	Fast	Causes hairy root disease of apples and other plants
R. radiobacter	A. tumefaciens + A. radiobacter + A. rubi	Peritrichous	59.5-63.0	+	-	Fast	Frequently produce galls on angiosperms
R. japonicum	R. japonicum + R. lupini + Cowpea miscellany	Subpolar	59.5-65.5	-	-	Slow	Nodulates many different legumes including one or more of Vigna, Glycine, Lupinus, Ornithopus, Centrosema, etc.

From: Graham, D-4.

been proposed. It is perhaps fortunate that the cowpea group, effective on most tropical grain legumes, seems to be widely distributed. Rhizobial population numbers may be maintained at a fairly high level by legumes in the native flora and, in many cases, are reasonably effective on sown legumes. However, as our knowledge grows it becomes increasingly evident that there are important differences among races within this broad group in terms of infectivity and N-fixing effectiveness. The matter of Rhizobium strain selection is treated elsewhere in this series (A-7).

In the case of inoculated legumes, the inoculum itself must be considered. All too often, inocula as received at the farm contain strains of bacteria inappropriate for the crop being sown or may even have no viable bacteria at all. Problems of inoculant quality are discussed in another paper (A-6). The matter of quality control is clearly a responsibility of the state; it is a very significant problem. If the farmer is using inoculum that is sound he must take reasonable precautions to assure that the seed are properly inoculated, discussed later, and that the inoculum and seed are handled in a way that will provide assurance that live bacteria will be present in adequate numbers at the time of germination.

Numbers of bacteria that are required for infection and nodulation can be estimated on a hypothetical basis but obviously cannot be estimated by looking at the seed. Assuming quality inoculum, the farmer can derive some assurance from the fact that if he can see the specks of inoculum on the seed the rhizobial population will probably be adequate.

Rhizobial survival under field conditions has been studied only

modestly. Many of the data in the literature have been derived from laboratory conditions, using temperate species.

The persistence of rhizobia in the field in the absence of an appropriate host is still a matter of conjecture, particularly under tropical conditions. Studies under temperate regimes indicate persistence of some species for from 10 to over 100 years, without the presence of a known host. In general, these data indicate that there is little correlation between numbers of bacteria in the soil and time expired since the host crop had last been grown or the number of times it had been sown. Survival seems to be a site-specific characteristic.

Certainly the presence of a compatible host tends to cause an increase in the population of those races of rhizobia that are infective; this does not imply that these strains are the most effective, though some natural selection must occur. Studies of competition among races, usually with the objective of trying to establish more effective rhizobial strains in the soil population, indicate that competitiveness, infectivity and ultimate effectiveness are not necessarily related. They also demonstrate the dismaying fact that strains that may be the most effective under laboratory conditions may be the least aggressive in establishing and maintaining an adequate population under field conditions.

Competition, possible toxicities, and actual predation among rhizobia and other members of the soil flora and fauna have also been suggested as important in affecting population numbers and persistence. Most of these studies have been conducted under laboratory conditions. The data

indicate that various species of the flora and fauna do interact to the detriment of the rhizobia. However, no conclusive data for field conditions have been published.

Several environmental (abiotic) soil characteristics have been identified as affecting survival of rhizobia or efficacy of the symbiosis. These are discussed in detail elsewhere in this series (A-2).

One such factor is the soil nutrient status. It has been well demonstrated that pH, nitrogen and phosphorus status of the soil can affect the bacteria. A reasonably well-defined minimum pH has been identified for several species of Rhizobium. These are indicated in Table 4. These values are not meant to mean that a pH lower than indicated is lethal; rather they indicate a pH above which growth and persistence are adequate, or below which they lose infectivity.

It has been postulated that some tropical rhizobia, perhaps because of natural selection, thrive at low pH levels and not at high. Following this hypothesis, the standard temperate zone recommendation of liming for legumes would be not only impractical, as it is at some locations, but also counterproductive. This possibility must certainly be taken into account if seed pelleting (see below) is being considered.

The other aspect of pH that has been of concern in enhancing biological nitrogen fixation has been the matter of saline and alkaline soils. Both occur between the Tropics with sufficient frequency to warrant mention of the problem. Though relatively little work has been done, the general conclusion may be drawn that high pH will limit host growth and survival more than it will affect the bacterium; though the end result, poor fixation, may be the same the cause does not appear to lie with the microsymbiont.

Table 4. pH below which *Rhizobium* does not grow or survive

Bacterium	Lower pH for growth or survival						
	Bryan (1923)	Fred and Davenport (1918)	Holding and Lowe (1971)	Jensen (1942)	Mendez-Castro and Alexander (1976)	Vincent and Waters (1954)	Graham and Parker (1964)
R. *meliloti*	4.6-5.0	4.9		5.3-5.5			4.5-5.0
R. *trifolii*	4.5-4.9	4.2	4.5	5.1-5.3	4.6	5.5	4.5-5.0
R. *leguminosarum*		4.7					4.0-5.0
R. *phaseoli*		4.2					4.0-5.0
R. *lupini*		3.15					4.0-5.0
R. *japonicum*	3.5-4.2	3.3			4.2-4.6		3.5-4.5
Rhizobium sp. (velvet bean)[a]		3.3					
Rhizobium spp.							4.0-5.0

[a]*Rhizobium* from the cowpea-type cross-inoculation group is designated for clarity as *Rhizobium* sp. and in parentheses the plant species from which the strain was isolated, if known.

From Lowendorf, A-2.

Aluminum (Al) and manganese have been frequently mentioned as being present in phytotoxic quantities in the soil solution of sub-tropical and tropical regions. This has led to their being investigated as potential constraints on rhizobial growth and persistence. Direct conclusive field evidence is still lacking. However, several pot experiments indicate that both Mn and Al may affect bacterial number, size and number of nodules, and effectiveness. These observations tend to be linked with alterations in root growth caused by high levels of soluble cations; a clear distinction has yet to be made. Tropical forage species have been rated for tolerance to soluble Al; the grain legumes have not been ranked though some work has been done with soybeans. The fact that tolerance has been demonstrated provides an alternative in those situations where toxicities may be suspected.

It is generally conceded that lack of phosphorus is the mineral factor that most often limits legume growth in tropical soils. Phosphorus is reputed to enhance seedling root growth, among other things. That this has a direct effect on the rhizobia has not been established. However, enhanced host vigor, particularly at the seedling stage, can be assumed to improve the possibility of satisfactory infection and nodulation.

The effect of nitrogen, mineralized in the soil or applied as fertilizer, seems to be mixed. For some legumes a light dressing of nitrogen (10-20 kg/ha) is recommended as a means of stimulating seedling growth prior to effective nodulation. Higher levels have been indicted as being inhibitory of nodulation and efficient biological fixation. This raises an interesting point of tropical crop management. Many seed-

ings are made with the beginning of the rains. It is common knowledge that the "spring flush" is caused by these same rains. The possibility that the increased mineral nitrogen available at the time of the flush may tend to inhibit rapid nodulation has not been investigated; it may be a significant factor.

It has been demonstrated that calcium, phosphorus, boron and lime are necessary for inoculation. Likewise calcium, phosphorus, boron, magnesium, sulfur and molybdenum are required for efficient nitrogen fixation. Copper, zinc and cobalt seem to play undefined roles in the process (Andrew, C-1 and Lowerdorf, A-2). The extent to which any or all of these nutrient elements affects the symbiotic relationship is probably highly site-specific and must be considered when evaluating the effectiveness of inoculation.

Because of prevailing climatic conditions, flooding and drought must be considered as they might affect the symbiotic relationship under tropical regimes. Soil temperature is mediated, in part, by moisture status and it, too, has been implicated in nodulation failures. Obviously, it is difficult to separate the two under tropical field conditions. Their effects have been partitioned in some studies. The data in Table 5 indicate some of the temperature maxima that have been estimated. The consensus seems to be that some moisture is beneficial in protecting the rhizobia at moderately high temperatures (40°) but that at very high levels (up to 100°) moisture can be deleterious. These studies also showed that the fast-growing bacteria (in general the temperate species) tend to be more susceptible to heat than the slow-growers (*R. japonicum* and *R. lupini*, for example) and that certain rhizobia

can be protected from high temperature effects by iron oxides and some clays.

Table 5. Maximum temperature for growth of Rhizobium in three studies.

Cross-inoculation group	Bowen and Kennedy (1959) Number of strains	Maximum range °C	Average	Ishizawa (1953) Number of strains	Maximum range °C	Mendez-Castro and Alexander (1976) Maximum range °C
Alfalfa	8	36.5-42.5	41.0	28	39-42.5	
Clover	9	31.0-38.4	33.2	5	35-37	38-39
Pea	2	32.0-32.7	32.3	8	35-37	
Cowpea group, etc.	68	30.0-42.0	35.4	49	32-42.5	
Lupine				9	32-33	
Soybean				10	32-35	42-43
Bean				8	35-37	

From Lowendorf, A-2

In studies on the effects of drought, soils have been dried to levels in the neighborhood of 5% moisture. In general, bacterial numbers decline with drying but the rate of decline and ultimate population level is not necessarily regulated solely by water content. The magnitude of the effect seems to be related, at least in part, to soil texture, i.e., in lighter soils the effect of drying is buffered as the clay content increases. Sugars also seem to provide some protection (See "Pelleting"). Slow growers tend to be more resistent than fast growers in a manner comparable to their response to high temperatures.

Finally, the evidence indicates that in response to either high or low temperature, and to drought, there is a marked species (strain?) response. Thus, the opportunity for both natural and controlled selection is present.

To the extent that it has been investigated, flooding seems to pose no threat to rhizobial populations. However, it seems reasonable to hypothesize that the effect of flooding, if any, would be associated with the water temperature and duration of submergence. Data now beginning to appear related to paddy-soybean rotations may shed some light on this question.

USE OF NITROGEN FERTILIZER WITH LEGUMES

Legumes are valued in agriculture for many reasons. Used as forage or pasture they increase the quality of the ration in terms of both protein and some minerals; used as cover crops or green manure they are reputed to enrich the soil because they tend to be more deep-rooted than the grasses and bring nutrient elements from lower horizons to the surface soil and because they increase the N content of the soil through symbiotic fixation; grown for food they enhance the diet qualitatively primarily with respect to the protein component but also in flavor and, perhaps, some minerals and vitamins. All of these reasons are based, to a greater or lesser degree, on the fact that legumes are capable of a symbiotic relationship that increases their nitrogen (amino acid, protein) content.

Cast in the simplest terms we are using the legume-*Rhizobium* symbiosis to replace fertilizer nitrogen. It is well-known that forage and pasture grasses, as well as most grasses grown for grain, can be managed to yield extremely well in terms of both protein content of the product and protein output per unit area. However, such yields are usually feasible only under highly intensive management and where the money value of the product more than covers the cost of the inputs, particularly N, to the system. If one were to set aside for the moment the matters of rising prices for N

fertilizers, increasing costs of production of those fertilizers, and threatened scarcity of some of the raw materials that go into their manufacture, it is reasonable to ask how legumes respond to fertilizer N as compared to that supplied through bacterial fixation.

With few exceptions, data from both forage and grain production trials indicate that legumes well fertilized with N yield little more than when they are adequately nodulated with an effective rhizobial strain. One reason for this may be the fact that sufficient inorganic N in the root zone suppresses both nodulation and the reduction of atmospheric nitrogen. For reasons not yet completely defined, the bacteria simply do not function if ammonia, nitrite or nitrate are available in the rhizosphere. Because they do not, the use of fertilizer N does not complement the symbiosis, rather it replaces it. Under these circumstances the supply of N to the plant may not be actually increased or, if some other factor such as energy supply is limiting, the plant will be incapable of assimilating the increased available nitrogen. In either case the gain will be either modest or nil.

The exception to this generalization is, of course, the case where the legume is not adequately supplied by the symbiosis. This may be caused by any of the factors mentioned earlier that can affect directly the nodulation and fixation capacity of the system. Numbers of bacteria may be limited, they may not be infective or effective on the legume grown, or the host itself may limit the process. Under such conditions, i.e., when some factor constrains either the host-rhizobium relationship or the bacteria but not the host itself, an agronomically significant response to fertilizer N may be anticipated. The increase may not be

economically attractive, however.

An example of host limitation is illustrated by recent work with Phaseolus vulgaris. Halliday and co-workers evaluated determinate bush- and indeterminate climbing-bean varieties. The determinate bush varieties are grown commercially, do not seem to respond to inoculation, and are routinely fertilized with nitrogen; the indeterminate climbers are "garden types," nodulate heavily and are rarely fertilized. The garden-type beans were of the same maturity as the bush beans; however, they had 12 times the nodule mass, 27 times the potential N-fixing capacity (by acetylene reduction), and yielded 59% more than the commercial-type bush beans. Further research indicated that the carbohydrates, the products of photosynthesis, were stored in markedly different fashion in the two bean types; the climbing beans had more soluble carbohydrates in the roots. The implications of these findings are worthy of consideration by anyone working in a developing agricultural economy; the contrast between a cultivar selected for machine harvest and commercial production and the native, or backyard, types should be noted by plant physiologists, breeders, and agricultural administrators. In selecting for uniformity of plant type and ease of harvest, have we selected against a process (symbiosis) that might permit more economical production?

Tables 6 and 7 show how the nitrogen fertilization of legumes may be evaluated from another perspective. Apart from the non-significant yield differences, that clearly indicate that symbiotic nitrogen fixation was worth at least 80 kg N/ha, these data on groundnuts (Arachis hypogea) and mung (Phaseolus aureus) indicate that inoculation is certainly best if

return per unit invested is the goal, as it might well be for a modest farmer. If marginal return is the criterion, as it might be for a commercial enterprise, the appropriate treatment might be quite different.

Table 6. YIELD OF GROUNDNUT PODS AS AFFECTED BY RHIZOBIA STRAINS AND NITROGEN LEVELS AND THEIR ECONOMICS

Treatment	Yield of pod (kg/ha)	Increased yield over control (kg/ha)	Extra expenditure over control (Rs/ha)	Profit or loss (Rs/ha)	Profit per invested rupee
Inoc. Strain "A"	2500	286	10.00	276	27.60
Inoc. Strain "B"	2605	391	10.00	381	38.10
Inoc. Strain "C"	2541	327	10.00	317	31.70
No Nitrogen	2214	--	--	--	--
20 kg N/ha	2274	60	55.00	5	0.09
40 kg N/ha	2599	385	110.00	275	2.50
60 kg N/ha	3109	895	165.00	730	4.42
80 kg N/ha	2333	119	220.00	-101	--

Sale rate of groundnut pods - Rs 100 per quintal

Table 7. YIELD OF MUNG AS AFFECTED BY RHIZOBIA STRAINS AND NITROGEN LEVELS AND THEIR ECONOMICS

Treatment	Yield of pod (kg/ha)	Increased yield over control (kg/ha)	Extra expenditure over control (Rs/ha)	Profit or loss (Rs/ha)	Profit per invested rupee
Inoc. Strain "A"	1356	283	10	273	27.30
Inoc. Strain "B"	1231	158	10	148	14.80
Inoc. Strain "C"	1270	197	10	187	18.70
No Nitrogen	1073	--	--	--	--
20 kg N/ha	1179	106	55	51	0.92
40 kg N/ha	1327	254	110	144	1.31
80 kg N/ha	1324	251	220	31	0.14

Sale rate of mung grain at Rs 100 per quintal
Cost of ammonium sulphate at Rs 53.00/quintal + Rs 2.00 application charges
Cost of inoculum at Rs 5.00/ha. Seed + Rs 5.00 treatment charges

Taken from P. Singh and S. D. Choubey, 1971. F-10.

The point should be made that with many types of farming units the question asked should not be "How well do legumes yield with N fertilizer?" but rather "How much N fertilizer does the rhizobial association replace?" As problems of cost and distribution mount, the replacement value of "free" fixation may quickly become the main criterion.

METHODS OF INOCULATING LEGUMES

The purpose of inoculating legumes, at seeding, is to ensure that an adequate number of the appropriate strain of rhizobia is present at the earliest time infection can take place. It simply involves mixing seed and bacteria to ensure early and intimate contact. This can be accomplished in many ways, some primitive and some relatively sophisticated; the goal is always the same.

The most crude methods of inoculation usually involved spreading trash or soil from a field where the same crop had previously been grown. This involved moving a tremendous mass of material. Perhaps the next step was to use soil from an old field to make a mud slurry which was scattered over the seed prior to planting. Though this was reasonably effective it had the disadvantage of spreading weeds and disease spores as well as ineffective strains of *Rhizobium*.

With the isolation of the causal organism, *Rhizobium*, in the late 19th century, microbiologists and agronomists began to devise more straightforward and efficient ways of achieving inoculation of seeded legumes. Current methods, apart from so-called preinoculation and granular materials applied with the seed, involve some form of cultured suspension of bacteria applied to the seed just before planting.

Nowadays, most common inocula consist of a moist suspension of

bacteria in some sterile carrier. Peat is commonly used but materials such as bagasse, coconut waste and rice hulls have been used.

Wet suspensions or cultures on agar are also used as inocula. Their value is limited because of problems in transport, storage and distribution. Lyophilized or oil-dried preparations are also available in some countries. Regardless of the type of inoculum used, some assurance of quality must be provided. (This subject is covered in paper A-6 of this series.) Some states and nations have quality-control laws; these normally guarantee a certain number of viable rhizobia of a given strain(s) at the time of manufacture. Many companies show expiry dates on their inoculum packages; these indicate a time after which the potency of the product is questionable. Unfortunately, each of these systems fails to provide assurance against improper handling after the product leaves the manufacturer. Poor storage conditions, particularly high temperature or direct exposure to sunlight, at any stage between processor and planting may result in inoculum that is completely valueless. While still in commercial channels, control of handling and storage procedures can be monitored by the State. Once in the farmer's hands, proper handling must be a matter of education.

Assuming that the farmer has access to peat-base type inocula of good quality chosen to match the crop he is seeding, the following are steps necessary for good inoculation:

1. The inoculum must be protected from high temperature and, particularly, from direct sunlight until the seed are in the ground.

2. Plan to inoculate the seed immediately before planting. As the bacteria on the seed dry, they die. Certainly no more than 48 hours should elapse before inoculated seed are used; if this is not possible it is best to reinoculate.
3. Avoid direct contact of inoculum or inoculated seed with caustic materials such as lime or potash, or with herbicides or fungicides, i.e., treated seed.
4. If treated seed is used it is advisable to "preplant" the inoculum, using an inert carrier such as moderately dry sand, cracked grain or sawdust.
5. To inoculate, select an area that is not in direct sunlight. The size of the area needed will depend on the quantity of seed to be inoculated; it may vary from a large tub to a smooth clean patch of ground perhaps two meters square.
6. Moisten the seed but do not wet them. The seed should be visibly moist but should not clump (if small seed) or drip, if large. Too much water can cause seeding difficulties or may actually damage the seed by softening the seed-coat prematurely. Mix thoroughly to ensure that all seed are moistened.
   An alternative method, not quite as effective, is to add a small amount of water to the inoculum to form a thin paste and to pour this over the dry seed. (If an agar- or liquid-base inoculum is used, this is the normal method.)
   In all cases cool, clean water should be used. Water causes the inoculum carrier to stick better to the seeds. It makes the inoculum go up to five times farther, particularly with large-seeded legumes.

7. The use of some kind of "sticker" is popular in many areas. Such things as corn syrup, sugar water, jarabe, gum arabic, various commercial cellulose compounds, maltose, and even kerosene and diesel fuel have been used. (It is essential that any suggested sticker be carefully checked to make sure that it contains no substance(s) that would inhibit germination or kill the bacteria.) A 10-15% sugar solution is suggested. Most substances mentioned enhance the amount of nodulation. The early success of the sugar and syrup stickers led researchers to believe that they provided a quick energy source for the bacteria. Such does not seem to be the case. However, sugar solutions do seem to confer a measure of drought resistance to the rhizobia. The ultimate decision on use of stickers and choice of material will depend largely on availability and cost. Their efficacy is well established.
8. Whether dry inoculum is shaken over moist seed or an inoculum paste (slurry) is added to dry seed, complete mixing is essential. This should be accomplished without handling the seed harshly; it should not be thrown or poured from waist height.
   It may be best to add half the required inoculum to the seed, mix and then add the second half and mix again, in order to guarantee adequate coverage.
   The seed should be examined. Each seed should have some black specks on it, indicating presence of the carrier (the bacteria cannot be seen).
9. Plant the seed on the same day as they were inoculated. Keep

them sheltered from heat and sun as much as possible. If a delay in planting of more than two days occurs, reinoculation is advisable. Because delay in seeding is undesirable and because thorough mixing is essential, it may be more practical to inoculate small quantities of seed for immediate sowing rather than one large amount that must be stored.

10. The amount of inoculum to be used is indicated on the container of most commercial products. [Nine ml. of slurry, containing 4.4 g of peat inoculum per kg of seed has been suggested for seed the size of soybean (Burton, C-4a).] A common unit in the United States is the bushel, a volume of 35 liters. Many inocula are packaged to be used on one bushel of legume seed. The area sown with this bushel will be governed by the seeding rate.

    If the inoculum is of dubious quality it may be wise to use double the recommended rate of application. Calculations in the U.S.A., based on alfalfa and clover inoculation, indicate a potential return of $200 for each dollar spent for inoculum (about 2000 lb of N fixed per package of inoculum costing $1 U.S.), so using double the recommended amount should still be economical under most circumstances.

11. If the inoculation should fail, that is if the young plants do not have nodules after about three weeks or if they are spindly and yellow, reinoculation may be attempted. Failures may occur if the soil is hot and dry at seeding or if the seed are planted shallowly and are exposed to sun and wind. Reinoculation can

be achieved by using an inert carrier, such as sand or even dry soil, or some of the same seed used earlier. Spreading the carrier, or planting the new, inoculated seed, will be most effective if done just before a rain or, if heavy dews are common, in the evening after the sun has gone down.

12. If conditions are normally harsh at seeding time and if inoculation failures are common, consideration should be given to the possibility of "preplanting" the inoculum (Point 4, above). Results obtained on light soils that tend to heat up to significant depths indicate that deep placement of the inoculum, i.e., below normal seeding depth, shows promise. Using this system, the young seedling-root makes contact with viable inoculum as it grows down to moist soil.
    Mulching or early irrigation, where feasible, is often helpful under such severe conditions.

PELLETING

The idea of pre-inoculating seed has been tried many times and many ways. Basically, the concept is that one should be able to add the appropriate rhizobia to the seed at the factory or main distribution point. This would eliminate many of the problems associated with inoculum distribution and storage and assure that the right strain was used with each cultivar.

The impregnation techniques, vacuum, etc., have generally proven to be unsatisfactory. The most popular current approach is that of pelleting. Pelleting is really more broad in concept than just preinoculation. The idea of enclosing the seed in a clump of lime, phosphate, etc., along with

an inoculum implies protection of the bacteria and seed, plus amelioration of adverse conditions in the soil adjacent to the seed. This is supposed to give both the seedling and the bacteria a better chance for a good start.

Because low pH has often been implicated in legume and inoculation failures under temperate conditions, limestone has been used to form the pellet. With the advent of the idea that tropical-legume rhizobia may not have pH or lime requirements like those of temperate species, plus the demonstrated need for adequate phosphorus levels, the use of rock phosphate, basic slag, etc., has increased. Other materials that have been used include talc, gypsum, dolomite, calcium silicate, bentonite, kieselguhr, bauxite, and blood.

Several stickers or adhesives have been used. The most popular seem to be gum arabic (15% water solution) and methyl ethyl cellulose (1% solution).

Results with pelleting and pelleting materials have been mixed. In areas where pH is very low or where soluble aluminum and manganese present problems the use of finely ground limestone may be justified. However, lime itself does have an alkaline reaction that is lethal for rhizobia. The fact that the alkalinity varies from source to source may explain some of the conflicting results that have been published. Therefore, it may be reasonable to consider rock phosphate as the material of choice unless a definite gain for limestone can be demonstrated.

Unlike inoculated seed, pelleted material can be stored for 2-12 weeks, depending on ambient conditions and legume species.

Pelleting permits seeding under somewhat more adverse conditions of

low pH, high temperature and dry soil than does simple inoculation. Pelleting may also provide some measure of protection against toxic substances in the seedcoats of such species as Centrosema pubescens and Trifolium subterraneum. Further, it has been suggested as a means of protecting the rhizobia from the fungicides and insecticides often used to pre-treat seed.

The process is relatively straightforward. Using an adhesive such as gum arabic (40%) or methyl ethyl cellulose (5%), one inoculates the seed. While still moist it is then tumbled in an appropriate container with the chosen coating material. This builds up a thick coating around the seed. Table 8 shows some appropriate quantities of sticker; it is undesirable to use too much because this may cause the seed pellets to clump in a sticky mass.

Table 8. A Guide to the Quantity of Material to use in Pelleting Seed

Purpose	Sticker concentration	Sticker to 1/2 pint water	Lime or rock phosphate coating (300 mesh)	Seed size*
Pelleting	Gum arabic 40%	4 oz.	8 lb.	15 lb. small 22 lb. small-medium
	Methyl cellulose 3-4%	1/3 oz. (1 rounded dessertspoon)	8 lb.	30 lb. medium 60 lb. large
Simple Sticker Inoculation	Gum arabic 15%	1 1/2 oz.	--	15 lb. small 22 lb. small-medium
	Methyl cellulose 2%	(1 level dessert-spoon)	--	30 lb. medium 60 lb. large

*Four basic seed sizes are recognized: small seed (white clovers, lotononis); small-medium seed (greenleaf desmodium, lucerne, stylo); medium seed (glycine, siratro, medics, subterranean clover); large seed (vetch, cowpea, Rongai dolichos, Leucaena).

From Diatloff. 1971. G-3.

EVALUATING THE RESPONSE TO INOCULATION

The final decision on the value of inoculation at any given site must be an economic one. Where inoculum is inexpensive relative to the expected gain, inoculation is often practiced as a form of "insurance." Farmers prefer to make the necessary investment as a means of avoiding the quantitative or qualitative loss that can be anticipated should the crop not yield its limit. Under tropical or sub-tropical conditions, where many of the crop legumes grown are compatible with the ubiquitous "cowpea miscellany" group of *Rhizobium*, the result may not be one of success or failure but rather of modest gains in infectivity of an introduced strain or effectiveness in fixing a greater quantity of nitrogen. Moreover it must be kept in mind that inoculation of legumes *per se* is not a panacea. It represents only one aspect of a total management program. Weed control, adequate fertilization, moisture stress (too much or too little), insect and disease depredations and crop or cultivar adaptation are other facets that should be considered in any specific environment. The professional agriculturalist must be constantly alert to the possibility that cause of the problem confronting him may not be apparent in the questions he is posed.

Under any circumstances, three questions must be asked if one is to evaluate a crop's response to inoculation:

1. Is an infective and effective strain of *Rhizobium* native to the site?
2. Does the added inoculum measurably improve the agronomic yield of the crop?
3. Is lack of N the factor limiting yield or is it something else?

These questions may be answered by the use of three field treatments:

1. Sow the legume as it has traditionally been handled. If a newly introduced species, sow according to the best available information but without inoculation.
2. Sow the legume as above but inoculate with the rhizobial strain or product to be tested (viability of the inoculum and adequate number of rhizobia/seed must be assured. A level of 10^6 bacteria per seed is assumed adequate); and
3. Sow as in (2) but fertilize with an appropriate quantity of nitrogen. The amount of N to use is uncertain but 100 kg of actual N per hectare should be sufficient. On very light soils or if heavy rains lead to excessive leaching or run-off, split applications with 15-20 kg N at seeding and the rest after the crop is well established may be desirable.

Possible responses to these three treatments are shown in Table 9. Their interpretation follows:

Table 9. Possible results from a 3-treatment inoculation trial to determine need to inoculate legume seed.[1]

Situation	Non-inoculated		Inoculated		Inoculated + Nitrogen	
	Nodulation	Plant Growth	Nodulation	Plant Growth	Nodulation*	Plant Growth
1	-	Poor	-	Poor	-	Poor
2	-	Poor	-	Poor	-	Good
3	-	Poor	+ E	Good	+ or -	Good
4	-	Poor	+ I	Poor	+ or -	Good
5	+ I	Poor	+ E	Good	+ or -	Good
6	+ I	Poor	+ I	Poor	+ or -	Good
7	+ E	Good	+ E	Good	+ or -	Good

- = plants not nodulated
+ = plants nodulated

E = effective nitrogen fixation
I = ineffective nitrogen fixation

*Plants may or may not be nodulated depending on their sensitivity to applied nitrogen.

1. No nodulation and plants unthrifty; small, pale-green uninoculated plants, indicating that native rhizobia, suitable for the test legume, are not present in the soil; similarly no nodules and no growth response from either the inoculated or inoculated plus nitrogen treatment indicating that some factor other than N,

[1] Under most conditions, nodulation should be checked about age 21 days. Subsequent checks may be in order, particularly if the rainfall pattern is erratic, but earlier evaluation may be premature.

e.g. phosphorus supply, is limiting plant growth.

2. No nodulation and poor growth of uninoculated and inoculated plants as above but healthy plants with added N; the inoculum strain or the quality of the inoculant supplied was unsatisfactory, or soil conditions were adverse for growth of the rhizobia and infection of the root. Nitrogen is limiting.
3. No nodulation and poor growth of the uninoculated plants as before, but effective nodulation of inoculated plants with growth similar to that in the plus-N treatment, indicating success of the inoculum strain in forming nodules and fixing nitrogen.
4. No nodulation and poor growth of uninoculated plants but inoculated treatment plants nodulated ineffectively and growth poor compared to the plus-N treatment, indicating that the inoculum strain was unsuited to the test legume.
5. Uninoculated plants poor and with ineffective nodules indicating nodulation by an unsuitable native strain; inoculated plants effectively nodulated and healthy demonstrating that the inoculum strain was both competitive with the native strains for nodule sites and effective in N-fixation with the test host.
6. Uninoculated and inoculated treatment plants nodulated ineffectively and poorly grown in comparison with the plus-N treatment, suggesting either that native rhizobia were ineffective and competitive for nodule sites, thus keeping out the inoculum strain, or that the inoculum strain was ineffective in N-fixation. It would be necessary in this case to use some strain-identifying technique such as serology or antibiotic resistant marker to

determine the proportion of nodules in the inoculated treatment due to the applied inoculum strain. (See appropriate paper in this series.)

7. Uninoculated plants with effective nodules and well grown compared with the other two treatments, indicating that native rhizobia suitable for the host legume are present in the soil. Obviously there has been no response to inoculation in this situation and nodules on the plants in the inoculated treatment would most likely be due to native strains. Again, this could be checked by serological or antibiotic marker identifying techniques. Under rare circumstances it is possible that the inoculated treatment in this situation could be nodulated and poorly grown, suggesting a highly competitive but ineffective strain in the inoculum.

   Modified from Date (C-1)

Response to inoculation is highly site-specific. Therefore, where little is known about the environmental conditions that affect symbiotic fixation it may be advisable to test at a maximum number of locations, at least as the first phase in an evaluation program. As the situations described above sort themselves out, more refined studies can be designed.

Halliday* has suggested an alternative 12-treatment field trial. In three prior steps he evaluates genetic compatibility and relative efficiency under laboratory conditions. Then, his field trial is:

1. Uninoculated, unfertilized control
2. Uninoculated control plus 150 kg P per hectare

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*J. Halliday, NifTAL, pers. comm.

3. Uninoculated plus 150 kg/ha each of N and P.

4-12. The three best strains from his earlier lab tests each used as inoculum in (a) lime pellet, (b) rock phosphate pellet, and (c) ordinary slurry.

This design seeks to answer more questions than the 3-treatment trial described earlier. Assuming a given level of resources, this type of test would be limited to fewer locations. On the other hand, if sufficient prior knowledge is available it provides significantly more information per location.

Plant response to inoculation can be evaluated in other, more specific, ways. Nodules may be counted and weighed, their position on the root system may be recorded, and their color may be checked. Finally, the nodules may be evaluated for energy (adenyl phosphate) or nitrogen-reduction (nitrogenase) potential. Total plant N, or the content of any plant part, can be measured by Kjeldahl analysis. Each of these has its place in evaluating response to inoculation and each is discussed in detail in accompanying papers (A-6 and A-7), but their value under most conditions should be very carefully considered. In many cases, if the magnitude of response to inoculation is the real objective, as compared to research into response mechanisms, the studies outlined above plus some measure of economic yield will suffice. More often than not, such a straightforward approach will provide evidence sufficient for farmers, agricultural professionals and policy-makers to plan an appropriate course of action.

Additional benefits to enhanced N-fixation might be the increased N content of the soil. Soil nitrogen reportedly increased 74% after the

addition of cowpea to a sorghum crop in India (H-4).

Finally, a qualitative response is sometimes achieved. Abu-Shakra (H-1) noted a 69% increase in soybean nodule dry weight, a 43% increase in grain yield (2090 vs 2990 kg/ha), an 8% increase in grain protein content, a 7% increase in methionine and a 4% decrease in cystine on inoculated soybeans in Lebanon; the latter two compounds are the amino acids most often in short supply in grain legumes, particularly soybeans. Similar qualitative responses have been reported by Kang (H-9).

CONCLUSIONS

Some basic principles concerning inoculation in the tropics are sound and well documented. They include: inoculation of tropical legumes will be beneficial if rhizobial strains are not present in the soil; if rhizobial strains are present and infection occurs but plant yields show nodulation to be inefficient, then inoculation with effective strains can certainly be beneficial; because of the many factors affecting inoculation and the rhizobia/legume symbiosis, one must be certain that other variables are considered before deciding inoculation is unnecessary. Some of the more important causes for failure to obtain increases in yields by inoculation include: 1) soils containing abundant supply of efficient nitrogen-fixing rhizobia (rarely prevails); 2) inoculant may be inefficient or non-viable; 3) environmental conditions may be unfavorable; 4) bacteria killed by direct contact with fertilizer, seed disinfectant, or toxic chemicals; 5) host or bacteria limited by lack of certain major (Ca, P) or minor (Mo, Zn, etc.) elements; and 6) presence in the soil of flora and fauna hostile to rhizobia.

It is believed that if microbiologists can select strains that are effective, competitive, and well adapted to certain climatic and geographi-

cal locations, inoculation of all legumes, grain and forage, should become widespread so as to save money on N fertilizer and give farmers a stable profit. It is recommended that the use of the on-farm tests be increased so that farmers (or researchers) can demonstrate for themselves the profit and stability one may obtain through the inoculation of legumes.

Though it somewhat overstates the case, a quotation from Smartt (B-5) serves to summarize the situation:

> "The whole question of nitrogen fixation in root nodules is one of great importance and complexity. It is quite apparent that there is scope for more extensive and extended investigation, particularly in the pulses. Considerable investigation has been carried out on the nodulation and nitrogen fixation of pasture legumes but much less on the pulses. The soya bean has been studied fairly extensively in the United States, more so than other important pulses. While the results from published work may indicate general guidelines as to what may be happening in the obscurer pulses, these must be accepted with some reservation."

Only fairly recently has intensive work begun on grain legumes of dietary importance in the tropics. Much of this is concentrated in the network of International Research Centers or at central national research facilities. All too little information reaches the small-holder level.

On the other hand, the small farmer is often faced with the dilemma of having the information available but of not being able to secure the necessary materials (inoculum) at the time, at a place, and at a price that he can afford.

The message is clear. Sufficient information, generated from work on temperate legumes and tropical forage species, is available to indi-

cate the tremendous potential of adequate inoculation technology if made available to tropical farmers. Site- and region-specific data are often lacking, difficult to locate or, unfortunately, of dubious quality. It behooves all agricultural professionals, from rural agent to central-government policy makers, to work toward assuring that, as legume production is intensified or as new legume species are introduced to a region, appropriate information about symbiosis, inoculation, and inoculum availability is provided as readily as is the seed.

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APPENDICES

APPENDIX I

Some commercially important tropical legumes.

Grain or edible parts

Arachis hypogaea (groundnut, peanut)
Cajanus cajan (arhar, pigeonpea, red gram, tur)
Canavalia spp (jack bean, sword bean)
Cicer arietinum (chickpea, dhal, garbanzo, gram)
Cyamopsis tetragonoloba (cluster bean, gram, guar)
Dolichos spp (horse gram, hyacinth bean, lablab)
Glycine max (soybean, soyabean)
Kerstingiella geocarpa (Kersting's groundnut)
Lablab niger (dolichos bean, field bean, hyacinth bean)
Lathyrus sativus (grass pea)
Lens esculenta (lentil)
Lotus tetragonolobus (asparagus pea)
Pachyrrhizus spp (yam bean)
Parkia filicoidea (locust bean)
Phaseolus spp (adzuki bean, black gram, common bean, green gram, haricot, lima bean, mat bean, mung, rice bean, scarlet runner bean, moth bean, snap bean, tepary bean, urd, yam bean)
Pisum sativium (peas)
Psophocarpus spp (four-angled bean, goa bean, winged bean)
Tamarindus indica (tamarind)
Vicia faba (broad- or horsebean)
Vigna spp (adzuki bean, asparagus pea, black gram, cowpea, green gram, mung bean, rice bean)
Voandzeia subterranea (bambarra groundnut, earth nut)

Forage, range, cover or green manure

Acacia spp (wattle)
Alysicarpus vaginalis (alyce clover)
Calopogonium mucunoides (calapo)
Centrosema spp (centro)
Clitoria spp (butterfly peas)
Crotalaria spp (senn hemp)
Desmodium spp (tick clover, greenleaf and silverleaf desmodium)
Glycine wightii (javanica)
Lespedeza spp (lespedeza, sericea)
Leucaena glauca
Lotononis bainesii
Lotus spp (birdsfoot trefoil)
Lupinus spp (lupin)
Moghania macrophylla (wild hops)
Phaseolus spp (phasey bean, siratro)
Pitecellobium spp
Prosopsis spp (algorroba, mesquite)
Pueraria spp (kudzu)
Sesbania spp

Stizolobium spp (bengal bean, velvet bean)
Stylosanthes spp (brazilian alfalfa, stylo, Townsville lucerne)
Tephosia spp
Trifolium spp (clovers)
Vicia spp (vetch)
Zornia spp

APPENDIX TABLE II

Legumes grouped by specific rhizobial inoculation strain (cross-inoculation groups).

ALFALFA GROUP

Common name	Scientific name
Alfalfa	Medicago sativa
Buttonclover	Medicago orbicularis
California bur-clover	M. denticulata
Spotted bur-clover	M. arabica
Black medic	M. lupulina
Snail bur-clover	M. scutellata
Tubercle bur-clover	M. tuberculata
Little bur-clover	M. minima
Tifton bur-clover	M. rigidula
Yellow alfalfa	M. falcata
White sweetclover	Melilotus alba
Hubam sweetclover	M. alba annua
Yellow sweetclover	M. officinalis
Bitterclover (sour-clover)	M. indica
Fenugreek	Trigonella foenum-graecum

CLOVER GROUP

Alsike clover	Trifolium hybridum
Crimson clover	T. incarnatum
Hop clover	T. agrarium
Small hop clover	T. dubium
Large hop clover	T. procumbens
Rabbitfoot clover	T. arvense
Red clover	T. pratense
White clover	T. repens
Ladino clover	T. repens (giganteum)
Sub clover	T. subterraneum
Strawberry clover	T. fragiferum
Berseem clover	T. alexandrinum
Cluster clover	T. glomeratum
Zigzag clover	T. medium
Ball clover	T. nigrescens
Persian clover	T. resupinatum
Carolina clover	T. carolinianum
Rose clover	T. hirtum
Buffalo clover	T. reflexum
Hungarian clover	T. pannonicum
Seaside clover	T. wormskjoldii
Lappa clover	T. lappaceum
Bigflower clover	T. michelianum
Puff clover	T. fucatum

PEA AND VETCH GROUP

Common name	Scientific name
Field pea	Pisum arvense
Garden pea	P. sativum
Austrian Winter pea	P. sativum (var. arvense)
Common vetch	Vicia sativa
Hairy vetch	V. villosa
Horsebean	V. faba
Narrowleaf vetch	V. angustifolia
Purple vetch	V. atropurpurea
Monantha vetch	V. articulata
Sweet pea	Lathyrus odoratus
Rough pea	L. hirsutus
Tangier pea	L. tingitanus
Flat pea	L. sylvestris
Lentil	Lens culinaris (esculenta)

BEAN GROUP

Garden beans, kidney bean, Navy bean, pinto bean	Phaseolus vulgaris
Scarlet Runner bean	P. coccineus (multiflorus)

LUPINE GROUP

Blue lupine	Lupinus angustifolius
Yellow lupine	L. luteus
White lupine	L. albus
Washington lupine	L. polyphyllus
Sundial	L. perennis
Texas bluebonnet	L. subcarnosus
Serradella	Ornithopus sativus

SOYBEAN GROUP

All varieties of soybeans.	Glycine max (Soja max)

APPENDIX TABLE II (continued)

Legumes grouped by specific rhizobial inoculation strain (cross-inoculation groups).

COWPEA GROUP

Common name	Scientific name
Cowpea	Vigna unguiculata
Asparagus-bean	V. sesquipedalis
Common lespedeza	Lespedeza striata
Korean lespedeza	L. stipulacea
Sericea lespedeza	L. cuneata
Slender bushclover	L. virginica
Striped crotalaria	Crotalaria mucronata
Sunn crotalaria	C. juncea
Winged crotalaria	C. sagittalis
Florida beggarweed	Desmodium tortuosum
Tick trefoil	D. illinoense
Hoary tickclover	D. canescens
Kudzu	Pueraria thunbergiana
Alyceclover	Alysicarpus vaginalis
(No common name)	Erythrina indica
Pigeonpea	Cajanus cajan (indicus)
Guar	Cyamopsis tetragonoloba
Jackbean	Canavalia ensiformis
Peanut	Arachis hypogaea
Velvetbean	Stizolobium deeringianum
Lima bean	Phaseolus lunatus (macrocarpus)
Adzuki bean	P. angularis
Mat bean	P. aconitifolius
Mung bean	P. aureus
Tepary bean	P. acutifolius var. latifolius
Partridge-pea	Chamaecrista fasciculata
Acacia	Acacia linifolia
Kangaroo-thorn	A. armata
Wild-indigo	Baptisia tinctoria
Hairy indigo	Indigofera hirsuta

The following legumes appear to require specific strains of legume bacteria for effective inoculation:

SPECIFIC STRAIN GROUP

Common name	Scientific name
Birdsfoot trefoil	Lotus corniculatus
Big trefoil	L. uliginosus
Foxtail dalea	Dalea alopecuroides
Black locust	Robinia pseudacacia
Trailing wild bean	Strophostyles helvola
Hemp sesbania	Sesbania exaltata
Kura clover	Trifolium ambiguum
Sanfoin	Onobrychis vulgaris (sativa)
Crown vetch	Coronilla varia
Siberian pea-shrub	Caragana arborescens
Garbanzo	Cicer arietinum
Leadplant	Amorpha canescens
Centro	Centrosema pubescens
Stylo	Stylosanthes guyanensis
Greenleaf Desmodium	Desmodium intortum