APPLIED BNF TECHNOLOGY

A Practical Guide for Extension Specialists

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INTRODUCTION

WHY TRAIN EXTENSION SPECIALISTS IN APPLIED BNF TECHNOLOGY?

This course is intended to train extension specialists in the applied technology of biological nitrogen fixation (BNF) of the legume-*rhizobium* symbiosis. While many manuals are available to train research workers in the techniques and science of legume BNF there are few training materials available that focus on the extension of BNF technology to farmers.

In most countries there are significant numbers of scientists trained to conduct BNF research. While critical to developing national BNF programs, quality research by itself does not ensure that farmers will benefit from BNF technology. It is technology transfer at the farm level which often limits the adoption and exploitation of this appropriate technology. Better communication at the extension and farm levels will, therefore, be required for farmers and national economies to fully benefit from BNF technology.

METHODOLOGY OF THIS TRAINING COURSE

Communication skills combined with a solid, realistic understanding of the technology are prerequisites for successful technology transfer. This course emphasizes training in both communication skills and basic information on applied BNF technology. Technology training emphasizes identifying and solving farmers' problems. This training/resource manual is not intended to be a comprehensive work on BNF. Sufficient material, however, is provided so this manual and accompanying materials will serve as a valuable reference for extension workers without access to library services. The writing has been done as simply and directly as possible to facilitate translation to other languages. Course materials emphasize information, demonstrations, and principles that are relevant and useful for extension agents in developing countries. The full course is designed for extension agents who are college educated and actively involved in improving other extension worker's technical skills and their ability to further transfer technology.

This course is based on the belief that despite the complexity of the biological and technical aspects of the BNF processes being taught, understanding the basic working principles of BNF technology is possible by non-scientists. The course is interactive with hands-on demonstrations and exercises, discussions, self evaluation, and review being an integral part of the leazrning process. Case studies are used for self-evaluation and review. Participants use their newly acquired knowledge of BNF to examine a series of realistic situations extension agents are likely to find in the field. Case studies challenge students to evaluate and diagnose problems and develop an action plan for their solution.

COURSE ORGANIZATION

Training materials are organized in modules. Each module begins with a summary of the key concepts and their relevance to applied BNF technology. A narrative follows which elaborates these key concepts using illustrative materials. Demonstrations of the key concepts augment the narrative and are an important learning tool. Instructions and details for performing demonstrations useful to other extension agents and farmers are provided to aid participants to develop their own training program.

The goal of this modular format is to assist course participants in developing programs at various levels of complexity and duration. All materials have been compiled and written toward ultimately benefiting audiences ranging from farmers to administrators.

The task of continuing to expand BNF technology by course participants will be made more effective by the addition to course materials of farmer handouts and training aids. A simplified summary of each module including illustrative material has been extracted and placed in a removable folder. These synopses will be useful for extension agent training and are conveniently used for reproduction or translation into local languages. Farmer handouts and illustrative material are designed to be easily reproduced by photocopying.

THE VALUE OF THE COURSE

Farmers will use BNF technology only (1) if they are aware of the potential economic benefit they can realize from its use and (2) if they can properly apply it in the field. Both these prerequisites for success require that farmers have confidence in the extension worker's ability and knowledge of BNF technology. We believe this training manual and course in applied BNF technology can make a contribution to increasing awareness and technology capability in institutions involved in technology transfer.

ACKNOWLEDGEMENTS

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HOW TO USE THIS TRAINING PACKAGE

ABOUT THE FORMAT

This training package is divided into three parts, the modules, training aids and the demonstrations. The primary goal of this course is to enable the participants to be prepared for presenting the materials to others. Therefore, we have included both technical resource information and simplified presentation materials.

ABOUT THE MODULES

The modules are written as a resource and background document. They contain a comprehensive overview of the legume-rhizobia biological nitrogen fixation symbiosis. Each participant will be given two additional books as further resource material. These are the FAO/NifTAL handbook primarily written by Dr. Joe C. Burton, Legume Inoculants and Their Use, and Methods in Legume-Rhizobium Technology, by Dr. Padmanabhan Somasegaran and Heinz Hoben.

A Summary and set of Key Concepts introduce the material contained in each module. Major topic areas are set apart for ease in following the material. These topic areas are listed in the Table of Contents. Illustrations and tables are liberally used to explain concepts and ideas. The final section of each Module gives a list of case studies and review questions as an additional learning tool and evaluation measure. This section is an opportunity for peers to evaluate the practical aspects of BNF technology and the opportunities and limitations of transferring the technology.

ABOUT THE TRAINING AIDS

A set of training materials is attached to each Module. Extension Specialists who take this course are expected to extend BNF technology through transfer of the knowledge they have gained. These materials consist of a lesson plan, enlarged copies of the Key Concepts, and selected visuals. Also included is a master copy and some samples of a simple to understand pictorial booklet for distribution to farmers. There is a 40-slide set supporting the concepts presented in each module. These training aids are encased in plastic for ease in reproducing and handling.

ABOUT THE DEMONSTRATION SECTION

Many demonstrations will be done to facilitate hands-on learning experiences. These demonstrations are appropriate for carrying out future technology transfer or for understanding important concepts. Complete instructions are given for carrying out selected demonstrations. The purpose and concepts given for each demonstration will be useful in deciding their possible applications. Not every demonstration is appropriate for every situation. Again, the emphasis of this course is on providing as much variety and flexibility as possible to participants in their future BNF technology transfer efforts.

ABOUT THE COURSE STRUCTURE

Giving course participants an overview of the practical applications of BNF is the determining factor in structuring this course. Thus, we begin with an introduction to nitrogen, legumes, and rhizobia. An explanation of their relationship, the legume-rhizobia symbiosis follows. A module on the production and use of inoculants provides the how-to aspect of BNF. Understanding the limitations and benefits of inoculation is the subject of the last two technical chapters. A primer on communication reemphasizes the importance of this aspect of BNF technology transfer. Finally, participants will blend their prior knowledge and professionalism with knowledge gained in the course to develop their own strategy for BNF technology transfer through a planning exercise and presentation practice.

MODULE NUMBER 1

NITROGEN AND TROPICAL AGRICULTURE

SUMMARY

As a key component of proteins, nitrogen is essential for all life. Nitrogen moves in a cycle from the atmosphere into the soil and plant system, through the food chain of animals and people, and back to the atmosphere. Most of the nitrogen available for cycling is in the atmosphere in a form that can only be used by certain bacteria. One group of bacteria called rhizobia can convert atmospheric nitrogen into a form that plants can use. Through a symbiotic association with members of the legume family, rhizobia convert atmospheric nitrogen to ammonia within root-nodules on the plants. This process is called biological nitrogen fixation (BNF). It is a crucial source of nitrogen for agriculture.

Nitrogen is the nutrient that is most often in short supply, limiting plant growth. For good crop production farmers usually need to add nitrogen to their soils, either as organic amendments, as inorganic fertilizer or by managing nitrogen fixation through the legume-rhizobia symbiosis. This third approach is the subject of this training manual.

KEY CONCEPTS

- Nitrogen is an essential element for all living things. It is a key component of proteins.
- Nitrogen moves through nature in a cyclic manner.
- Specialized bacteria can convert nitrogen from the atmosphere into a form that plants can use. This process is called biological nitrogen fixation (BNF).
- Through a symbiotic relationship with legume plants, rhizobia provide the plant with "fixed" nitrogen, which the plant uses for its growth.
- Inorganic nitrogen fertilizer is produced by chemical nitrogen fixation, an expensive process requiring nonrenewable energy inputs.
- Biological nitrogen fixation in the rhizobia-legume symbiosis is an inexpensive, valuable management option for farmers.

NITROGEN IN NATURE

All living things need nitrogen (N), because it is the key component of amino acids, the building blocks of proteins. Almost 98% of all nitrogen is bound-up in primary rocks in the earth, but this nitrogen is not available to cycle through plants, animals, soil, and air. The remaining 2% of `unbound' nitrogen cycles above and through the earth. It is distributed in the following proportions:

- In the atmosphere.....99.96%
- In soil humus.....0.02%
- In sea-bottom organic compounds.....0.01%
- In living plants, animals, and microorganisms.....0.005%

From S.R. Aldrich, 1980. Nitrogen in Relation to Food, Environment, and Energy. Special Publication 61. Agricultural Extension Station, University of Illinois (USA).

The air is almost 80% nitrogen gas (N_2), but plants and animals cannot use nitrogen directly from the air to make protein. Yet the amount of nitrogen present in all of the world's soil humus and living plants and animals is extremely small—far too little to support the continued productivity of natural or agricultural systems. These systems depend on atmospheric nitrogen that is converted, or **fixed**, into a chemical compound that plants can use.

The manufacture of nitrogen fertilizers involves chemical fixation of nitrogen. In nature, a small amount of nitrogen is converted through lightning discharges and volcanic emissions, but most nitrogen fixation is due to certain types of bacteria and other microorganisms. The process by which they convert atmospheric N_2 into a form that plants, and ultimately animals, can use is called biological nitrogen fixation or BNF.

Once N_2 from the air is fixed, it moves through nature in a series of interlocking, cyclic reactions. First it is transformed to NH_3 and then to protein. Protein accumulated by plants passes through the food chain to animals and people. Residues returned to the soil by plants, animals, and people also contain nitrogen as protein. These residues are decomposed by bacteria in the soil, in a process called mineralization.

The mineralization process results in the release of nitrogen as **ammonium** (NH_4). Some ammonium is absorbed by plants through their roots, but most is converted by other soil bacteria to **nitrate** (NO_3). This nitrate moves easily through water in the soil and is absorbed by plants. Within the plant, the nitrate (or ammonium) is converted into protein. Nitrate can also be changed back into nitrogen gas (N_2), permitting its return to the atmosphere. This conversion, called **denitrification**, is done by other bacteria found in soil and water.

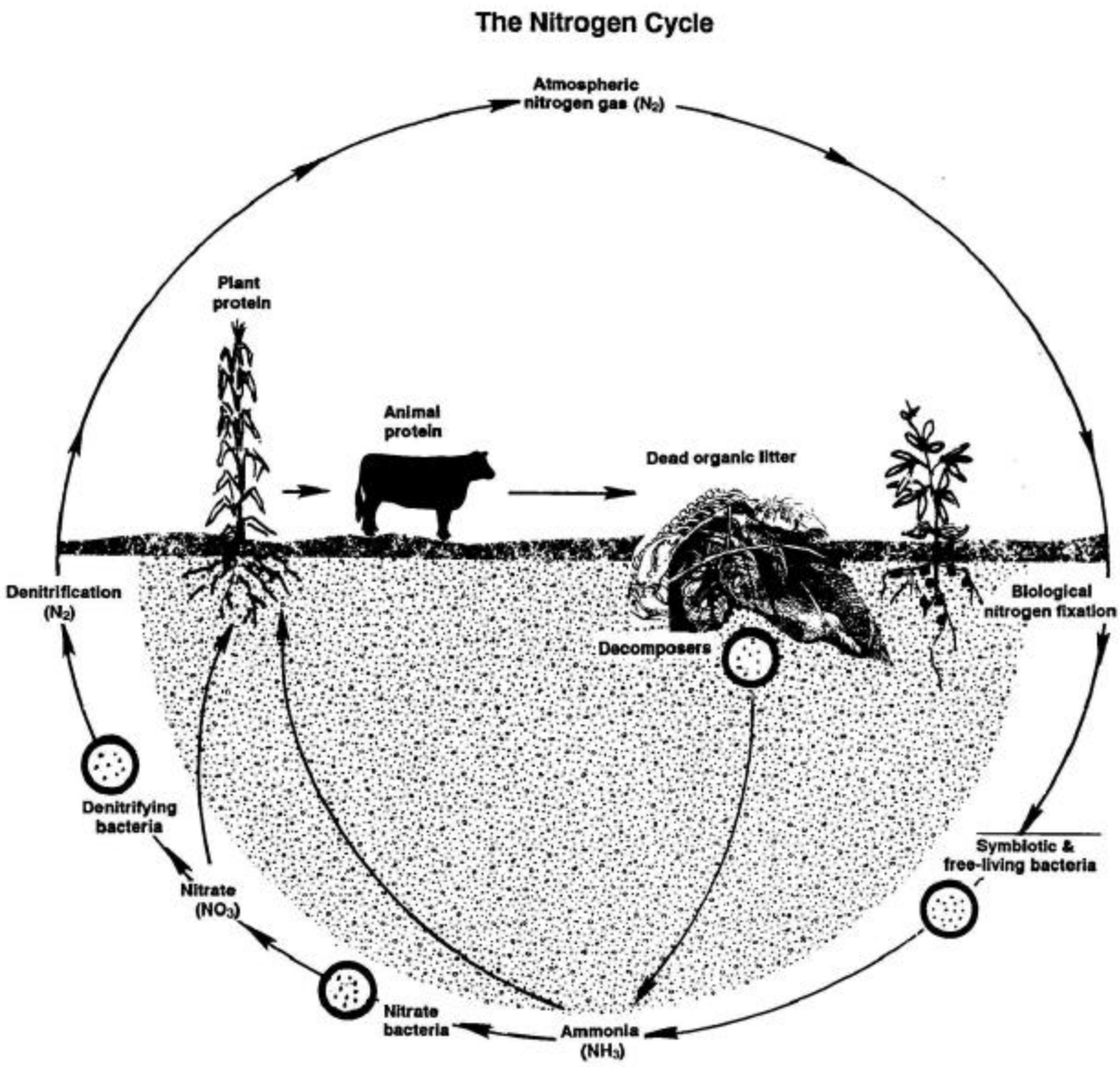


Figure 1-1. A simplified nitrogen cycle.

In summary, atmospheric N_2 is converted by specialized bacteria working in symbiosis with plants into NH_3—a process called biological nitrogen fixation. The plants then convert the NH_3 into proteins. Eventually these proteins enter the soil as residues. Other specialized bacteria convert the nitrogen in these residues into NH_4 and NO_3, which can be taken up by plants. Some NO_3 is converted back to atmospheric N_2.

NITROGEN IN HUMAN NUTRITION

Nitrogen is a key component of all proteins, necessary for the growth, maintenance, and repair of body structures. The bones, muscles, skin, and other solid parts of the body are made up largely of proteins. Proteins also provide energy and help protect people against disease and malnutrition. We cannot synthesize our own proteins: We must obtain them from our food, either from animal products (cheese, eggs, meat, and milk) or from plant products (legumes, grains, nuts, and vegetables).

Table 1-1. Yield and protein content of certain tropical food crops.

Crop	Estimated Yield[a] (kg/ha)	Protein Content[a] (%)
Legumes		
Soybean	2,800	38.0
Lima Bean	3,200	25.0
Cowpea	1,800	25.0
Peanut	1,600	26.0
Winged bean	1,400	31.0
Chickpea	2,500	20.0
Mungbean	900	24.0
Root Crops		
Sweet Potato	20,000	1.3
Potato	15,000	2.0
Cassava	20,000	1.2
Cereals		
Rice	5,000	7.5
Maize	4,000	9.5
Sorghum	3,500	10.1

For legumes and cereals, the yields and protein content are for harvested seed. From R.A. Luse and P.E. Okwuraiwe, 1975. Role of legumes in tropical nutrition, pp. 98-100. In R.A. Luse and K.O. Rachie (eds.) Proceedings of the IITA Collaborators' Meeting on Grain Legume Improvement. Ibadan, Nigeria: International Institute of Tropical Agriculture

Legumes Are Rich in Protein

In many countries, legumes are a main source of dietary protein. The legumes are combined with cereals or grains for a complete protein balance. Although legume crops commonly have lower yields than cereal or root crops, their protein concentration is much higher, largely because of biological nitrogen fixation. Even with lower yields, farmers can obtain more protein from legumes than from cereal or root crops.

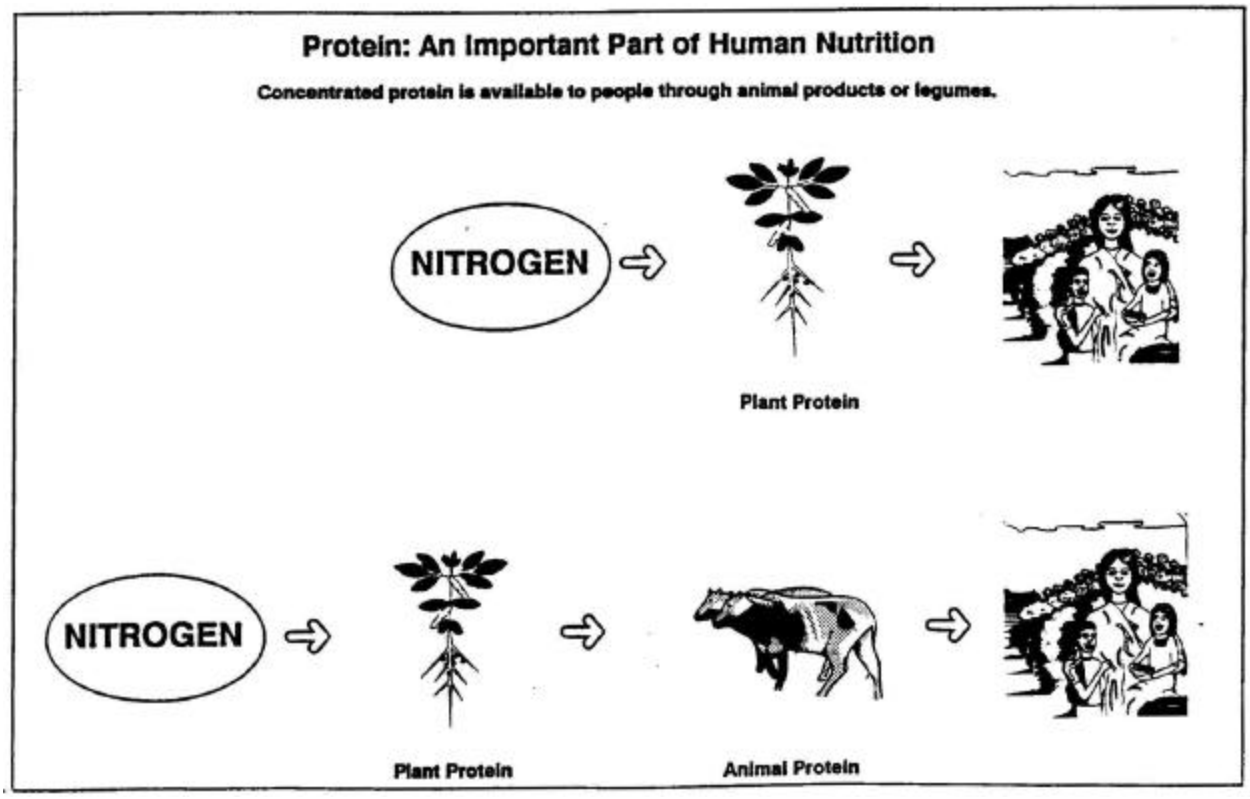


Figure 1-2. Protein: An important part of human nutrition

NITROGEN IN AGRICULTURE

In plants and soils, nitrogen occurs mainly in organic forms such as protein and soil humus. The amount of nitrogen in each form varies: Forests, for example, hold most of their nitrogen in plant biomass and litter, whereas grasslands and croplands usually hold most of their nitrogen in soil humus. In addition to these forms of organic nitrogen, ammonium and nitrate contribute a small percentage of the total nitrogen in plants and soils.

Farm Management is Important in the Nitrogen Cycle

Nitrogen is the most commonly deficient plant nutrient. Good management of crops and soils affects the amount of nitrogen available for plant growth. How much nitrogen a soil naturally supplies to plants depends on the rate of nitrogen mineralization, which in turn depends on the amount of organic nitrogen available and on conditions of temperature, water, and aeration. Fortunately, management of temperature, water and aeration to benefit crop productivity also promotes the activity of the bacteria responsible for nitrogen mineralization.

Soil nitrogen can be increased by adding organic matter taken from somewhere else, by growing green manure crops to till into the soil, or by conserving and incorporating crop residues. In the long term, soil nitrogen depends largely on plant growth, which is the source of most soil organic matter. This generalization leads to the simple rule that nitrogen fertility is maintained most effectively by residue conservation combined with agricultural practices that produce the highest yield and greatest biomass.

Do Soils Have Enough Nitrogen for Crops?

Even with high soil organic matter, nitrogen mineralization is rarely fast enough to meet the nitrogen demands of vigorously growing crops. Farmers therefore supplement the soil's ammonium or nitrate, either directly by adding inorganic nitrogen fertilizers or indirectly by adding organic materials or promoting biological nitrogen fixation.

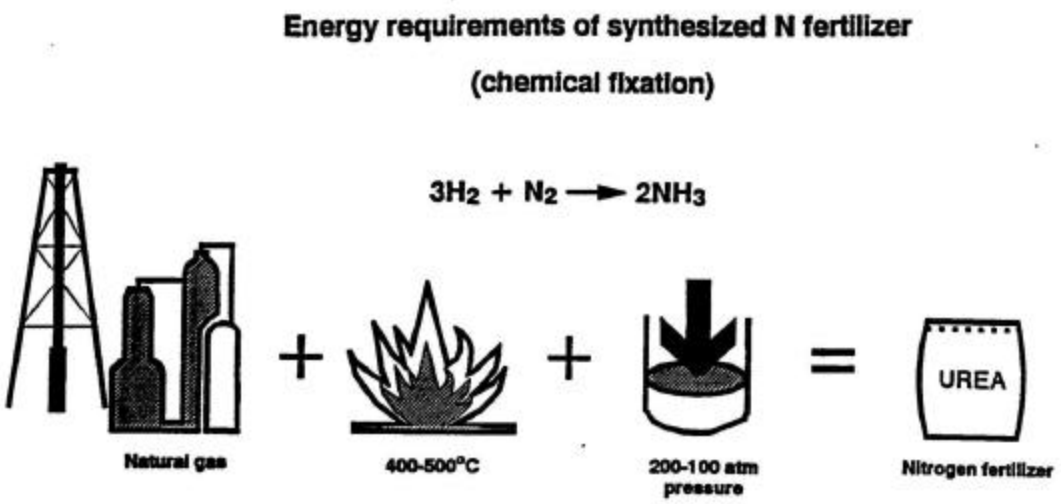


Figure 1.3. Energy requirements of synthesized N fertilizer

Inorganic Nitrogen Fertilizer

Inorganic nitrogen fertilizer was first synthesized in 1921. It is produced industrially by chemically fixing the N_2 gas in the air to form ammonium. This requires energy-expensive components and processes, which accounts for most of the fertilizer's cost. The use of synthetic nitrogen fertilizer has grown tremendously since its introduction, representing a truly significant alteration of the nitrogen cycle. It is estimated that the annual amount of N_2 now fixed industrially—about 60 to 80 million tons—equals the amount fixed by biological nitrogen fixation.

BIOLOGICAL NITROGEN FIXATION (BNF)

Bacteria are the only organisms capable of biological nitrogen fixation (BNF). There are bacteria that carry out this process on their own, and others that fix nitrogen while housed within the root system of plants (**symbiotic nitrogen fixation**). While symbiotic nitrogen fixation is associated with several plant families, the fixation associated with **legumes** has the most benefits for agriculture. Symbiotic nitrogen fixation benefits both the bacteria and the plant, and can be managed to benefit entire agricultural systems. More specific information about symbiotic BNF is given in **Module 4**.

Many Familiar Crops are Legumes That Support BNF

The nitrogen-fixing symbiosis most beneficial to agriculture occurs between a type of soil bacteria called **rhizobia** and the legume plant family (Leguminosae). The legume family is large: It includes many familiar plants such as peanuts (groundnuts), soybeans, green beans, mungbeans, faba beans, cowpeas, chickpeas, lima beans and others, plus many pasture and tree crops. When these legumes meet the right rhizobia, they produce small growths on their roots, called **nodules**, where the rhizobia grow and fix nitrogen. The plant feeds the rhizobia the energy that they need for growth, and in return the rhizobia give up their fixed nitrogen to the plant, which converts it to protein. If other growth conditions are adequate, this process of biological nitrogen fixation can supply the legume with most or all of the nitrogen it needs for optimal yield, without addition of nitrogen fertilizer.

Different Rhizobia for Different Legumes

Rhizobia and legumes have evolved together over many millennia to become fairly specialized. For example, the rhizobia that cause nodules and fix nitrogen with faba beans cannot do so with soybeans, and vice versa. Rhizobia may be present in the soil but may fail to fix enough nitrogen for a legume plant, either because there are too few rhizobia or because they cannot fix nitrogen with that particular legume species. The appropriate rhizobia can be added to the seed before planting or to the seed bed. The process of adding rhizobia is called **inoculation**. Rhizobial inoculants are produced commercially and packaged with a carrier material such as peat. They are commonly available in countries where legumes are an important part of the agricultural production system. **Module 3** discusses the specific rhizobia required by different legumes, and **Module 5** explains the use of inoculants.

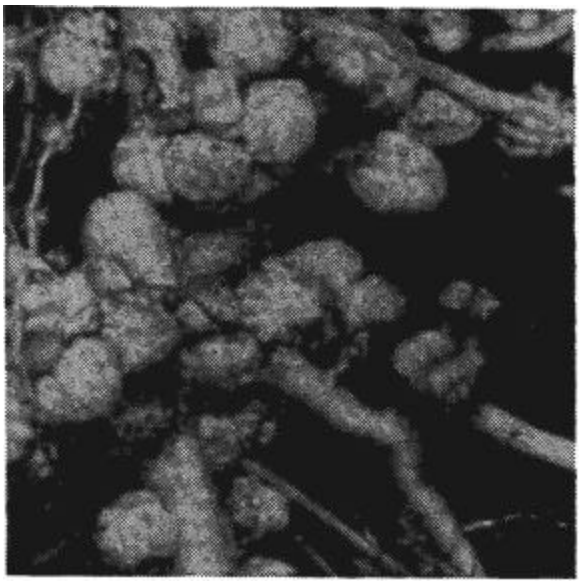


Figure 1-4. Root nodules of soybean (*Glycine max*) nitrogen fixing bacteria (rhizobia) are located within the nodules.

How Do Rhizobia and Legumes Get Together?

Rhizobia present in the soil or introduced by inoculation begin to multiply on the surface of the young roots of an emerging legume plant. They enter the roots through root hairs. After about two weeks, small bumps appear on the root. These bumps eventually become larger and mature into fully functional nodules. The nodules produce nitrogen for the legume throughout the growing season, with peak activity usually at about the time of flowering. The number, size, and activity of the nodules depend on several factors—most importantly on the number and effectiveness of the rhizobia present at planting plus all the environmental and management factors that influence the general growth and vigor of the plant. When the plant is harvested or dies, the nodules wither and fall off the roots. As they decay in the soil, some of the rhizobia are released. If soil fertility, temperature, and moisture conditions are favorable, the released rhizobia may survive in sufficient numbers to inoculate new plants of the same legume species the following season.

THE NEED FOR BNF IN AGRICULTURAL SYSTEMS

Since nitrogen is commonly the most important nutrient limiting crop-production systems, it must be managed properly to obtain good yields. This can be achieved by adding nitrogen fertilizer or manure, or through BNF in the legume-rhizobia symbiosis. Fertilizers are expensive, both in terms of the energy used to produce them and the cost to farmers. Manure may not be available in adequate amounts, and application can be difficult and labor intensive. BNF through inoculation of legumes offers an alternative that is fairly simple and inexpensive and over the years provides a large return on the time and money invested. The introduction and maintenance of effective rhizobial populations in a farmer's field are valuable techniques for the production of nitrogen for legume crops.

Can Legumes Provide Nitrogen to Other Crops?

Nitrogen can be added to cropping systems by growing legumes as rotational crops, rotational pastures, green manures, intercrops, or range plants. The benefits that accrue from productive legume crops can extend to subsequent non-legume crops. The nitrogen in residues from the legumes becomes available to other crops through the process of nitrogen mineralization. The amount of nitrogen provided depends on several factors: These will be discussed in more detail in **Module 4**. Nitrogen fixation stops if there is already too much nitrogen in the soil or if plants are growing poorly due to some other factor. Nitrogen fixation by legumes is most effective in soils with low organic matter when legumes grow vigorously and are well nodulated.

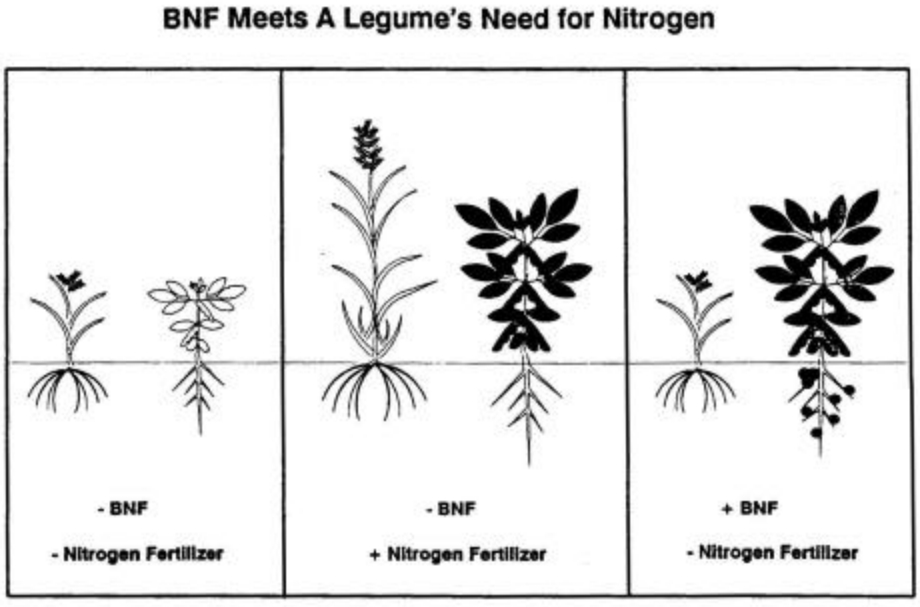


Figure 1-5. BNF meets a legume's need for nitrogen

BNF Has Other Advantages Compared to Fertilizer Nitrogen

It is easy to add too much inorganic nitrogen fertilizer to a cropping system. Crops usually use only one-half or less of the fertilizer nitrogen applied. Nitrogen fertilizer that is not used by plants can cause water pollution. Rain and irrigation water carry the unused fertilizer into streams or lakes, where the nitrogen compounds stimulate growth of algae and other water plants. Excess nitrate (NO_3) can leach into drinking water supplies, and high levels are toxic to humans.

Legumes can receive their nitrogen from BNF, without the problems associated with nitrogen fertilizer use. In addition, the cost of nitrogen fertilizer can be too high for many farmers—rhizobial inoculant costs much less.

REVIEW AND DISCUSSION

- What are the common legume crops grown and eaten by farmers in your area?
- Have you observed nodules on these legumes?
- Are these legumes commonly inoculated with rhizobia?
- How will the market price of oil/gas influence the cost of nitrogen fertilizer? Will these changes influence cropping systems?
- Can you define a typical cropping system in your region and develop a management strategy that improves the nitrogen balance of the system? Include realistic inputs and outputs of nitrogen in the system.

SUGGESTED LESSON PLAN FOR MODULE 1

TIME: One Hour +

This lesson can be taught in a very short time, especially if participants are trained in agriculture or a related field.

OBJECTIVES:

Understanding the important role of nitrogen in nature and in human nutrition. Knowing that BNF is an important part of nitrogen cycling in the environment

MATERIALS:

Demonstrations D1/1 and D1/2

Training Aids for Module 1

STEPS:

1. Assemble materials for demonstrations. Display key concepts and other visual materials.

2. Using the Module as a basis, organize your talk according to the background and skill level of your audience.

For example, begin with the broad theme of nitrogen in the environment, and move toward the more specific themes of nitrogen in agriculure, Biological Nitrogen FIXation, and the role of nitrogen in human nutrition.

3. Be sure to begin by asking questions to get people involved in the learning process and to become familiar with their knowledge level. This step is important in every module, as well as asking questions at the end of the presentation to check learning, because each module presents information that builds knowledge necessary to understand the following modules. The case studies at the end of each module should help with this.

KEY CONCEPTS

Nitrogen is an essential element for all living organisms. It is a key component of proteins.

Nitrogen moves through nature in a cyclic manner

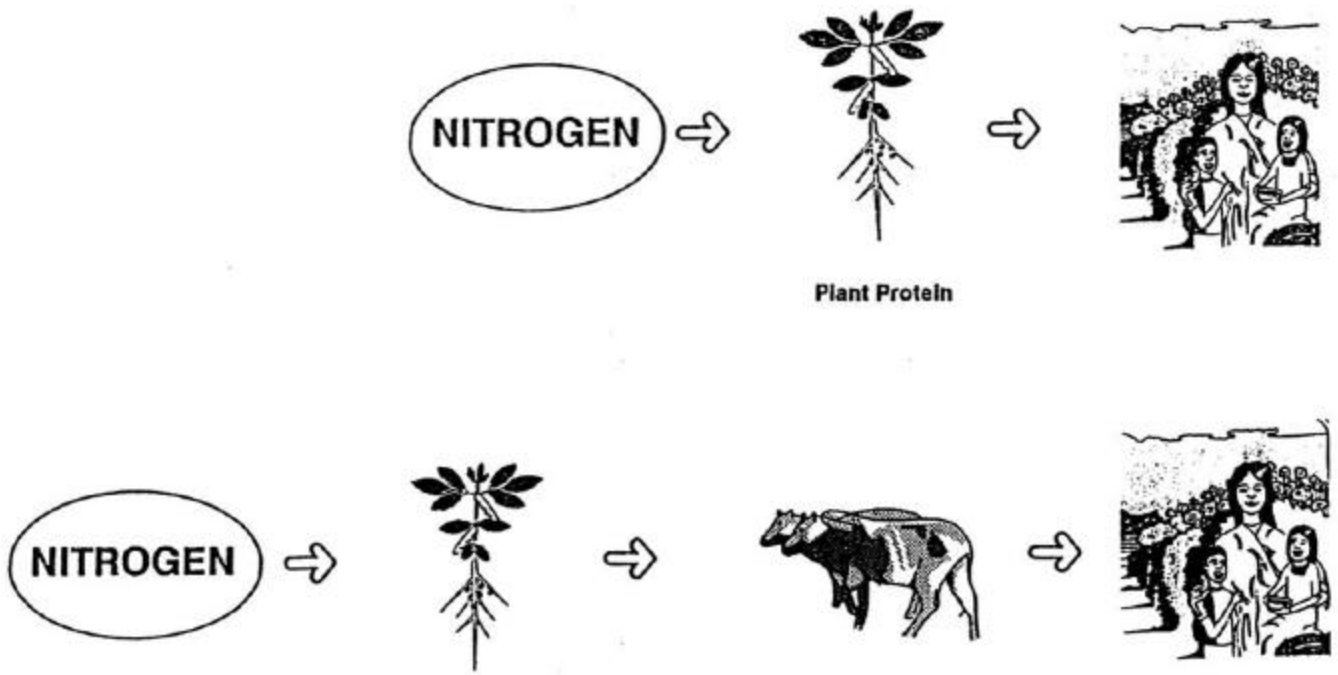
Bacteria are able to convert atmospheric N to ammonia in a process called biological nitrogen fixation (BNF).

In the rhizobia-legume symbiosis, rhizobia provide the plant with fixed N, which the plant uses for its growth. Inorganic N. fertilizer is produced by chemical nitrogen fixation, requiring nonrenewable energy inputs.

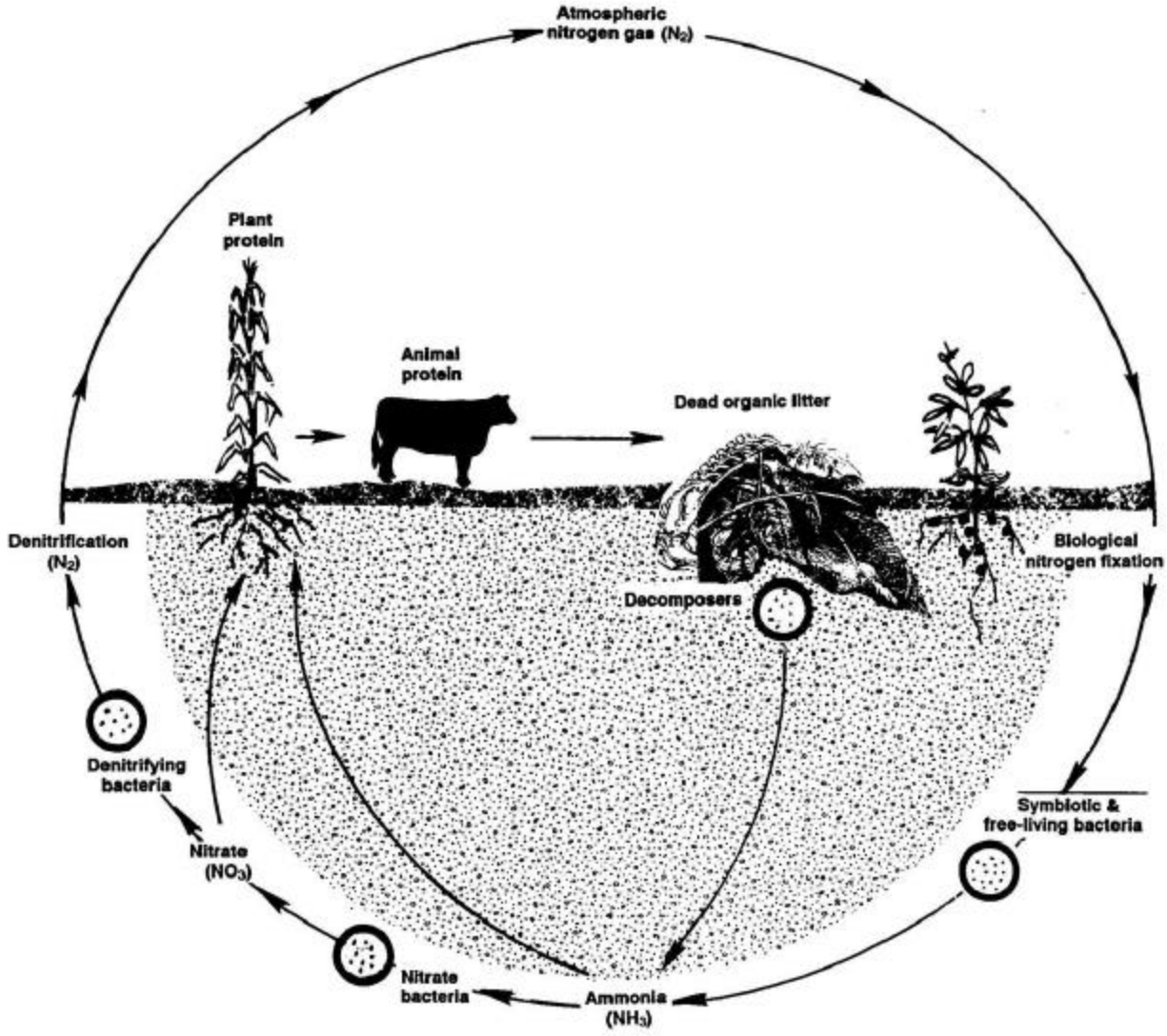
Biological Nitrogen Fixation in the Rhizobia-legume symbiosis is an inexpensive, valuable resource option for a farmer.

Protein: An Important Part of Human Nutrition

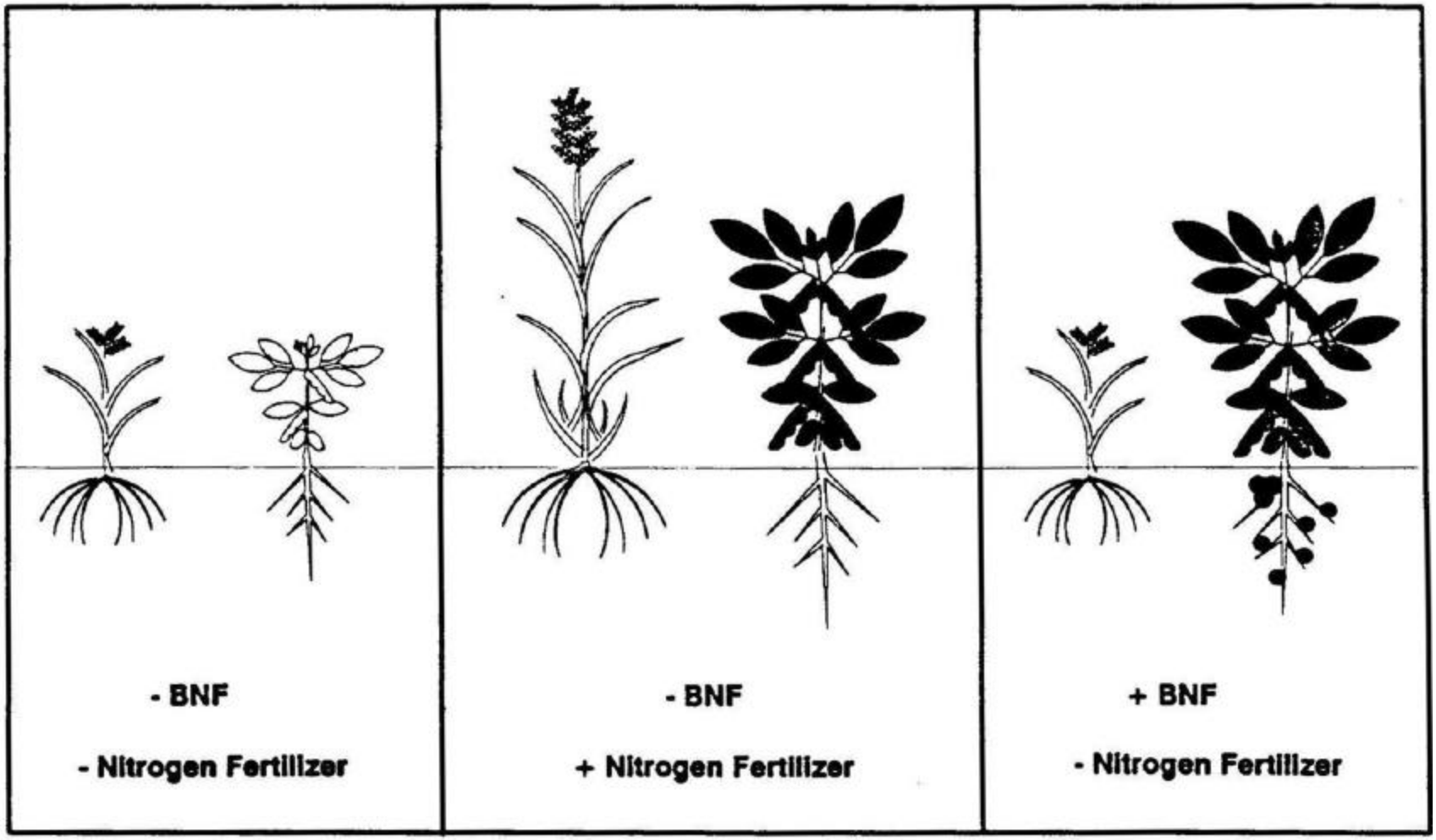
Concentrated protein is available to people through animal products or legumes.



The Nitrogen Cycle



BNF Meets A Legume's Need for Nitrogen



MODULE NUMBER 2

LEGUMES AND THEIR USE

SUMMARY

Legumes and cereals are the two most important flowering plants used in agriculture. Legumes are useful as human and animal food, as wood, and as soil-improving components of agricultural and agroforestry systems. This module summarizes the history of legumes and the discovery of their role in the legume/rhizobia symbiosis. The four subfamilies of the legume family, Leguminosae, are discussed. Drawings of legume flowers, leaves, and pods should help identification in the field. A comprehensive table gives the subfamilies, species, common names, habits, uses, and geographic areas of some important legumes.

KEY CONCEPTS

- Legumes are among the three largest families of flowering plants and have a long history of use in agriculture.
- Some, but not all, legumes produce nodules in symbiosis with bacteria.
- Legumes belong to the family Leguminosae, (also known as Fabaceae) which consists of four subfamilies, the Papilionoideae, Caesalpinoideae, Mimosoideae, and Swartzioideae.
- The most dependable way to identify the legume subfamilies is by examining the plants' reproductive structure.
- Legumes have multiple uses.

THE LEGUMINOSAE

Legumes are among the three largest families of flowering plants. The flowering plants of greatest importance to world agriculture belong to the orders Gramineae (cereals and grasses) and Leguminosae (legumes or the bean family). The Leguminosae consist of about 750 genera and 19,000 species of herbs, shrubs, trees, and climbers. This large family is divided into four subfamilies—the Mimosoideae, Caesalpinoideae, Swartzioideae, and Papilionoideae. The Swartzioideae is a small subfamily of about 80 species and relatively unimportant economically.

People have been growing legumes as crops for 6000 years. In Switzerland, the lake dwellers who lived between 5000 and 4000 B.C. cultivated peas (*Pisum* sp.) and a dwarf field bean, both legumes. In China, farmers began cultivating soybeans between 3000 and 2000 B.C. Legumes like lentils were also components of the cropping systems of ancient Egypt, and faba beans are mentioned in the Bible.

NODULATION AND BNF

Discovery of Nodulation and BNF

Farmers have long appreciated the value of legumes for improving and sustaining soil fertility. In the nineteenth century, Lawes and Gilbert (England) showed that legumes improve soil fertility by adding nitrogen to the soil. Hellriegel and Wilfarth (Germany) showed that pea plants gain nitrogen only in the presence of soil microorganisms and that the legumes' root nodules are intimately involved in the process. In 1887, Marshall Ward (USA) showed that root nodules are formed only in the presence of soil bacteria. Finally, in 1888, Beijerinick (Holland) isolated the nitrogen-fixing bacteria from nodules and from soil. The generic name given to these bacteria was *Rhizobium.*

Does Nodulation Occur in All Legumes?

The discovery of BNF in some legumes, with its dramatic potential benefit to agriculture, created a strong incentive among scientists worldwide to investigate the extent of nodulation in the Leguminosae. **Table 2-1** gives an estimate of the incidence of nodulation, and hence likelihood of BNF, in the three important legume subfamilies.

Although nodulation has not been investigated in many species, **Table 2-1** suggests that most of the species in the Caesalpinoideae subfamily do not produce nodules. Common genera in this subfamily that do not produce nodules are *Caesalpinia, Cassia*, and *Bauhinia*. The highest incidence of nodulation appears to be in the Papilionoideae subfamily, followed by the Mimosoideae.

Table 2-1. Nodulation in the subfamilies of the *Leguminosae*.

		Number of species reported		
Subfamily	Estimated number of species	Nodulated	Not nodulated	Total
Mimosoideae	2,900	351	37	388
Caesalpinoideae	2,800	72	180	252
Papilionoideae	14,000	2,416	46	2,462
Total	19,700	2,839	263	3,102

From O.N. Allen and E.K. Allen, 1981. In Leguminosae: A Source Book of Characteristics, Uses, and Nodulation.

LEGUME IDENTIFICATION

Plants in the Leguminosae family have characteristic leaves and pods that help identify them as legumes. The leaves are usually alternate (**Figure 2-1**: 14) and compound (**Figure 2-1**: 8, 9, 13, 14, and 15). They may be pinnate (**Figure 2-1**: 9) or trifoliate (**Figure 2-1**: 12). All legumes have similar fruits, called `pods', as shown in **Figure 2–2**. Within the Leguminosae, particular subfamilies and species can only be distinguished reliably by an examination of their flowers. For accurate identification of legume species in the field, consult a botanist or send a specimen to the national arboretum in the country where you work.

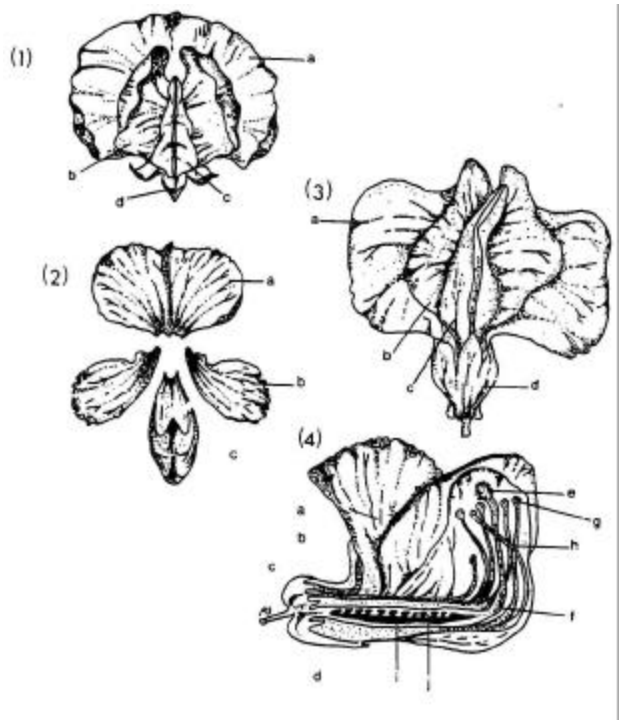


Figure 2-1. Subfamily Papilionoideae.
1. front view of flower of *Pisum sativum* (pea); 2. petals of *P. sativum*; 3. flower of *Psophocarpus tetragonolobus* (winged bean);
4. flower of *P. tetragonolobus* in longitudinal section. a-posterior or standard petal; b-lateral petal; c-keel petals (carina); d-sepals; e-stigma; f-style; g-anther; h-filament; i-ovary wall; j-ovule.

Figure 2-2. Subfamily Caesalpinoideae.
1. bud of *Cassia* sp.; 2. flower of *Cassia* sp.; and 3. longitudinal section through flower of *Delonix regia* (Flame of the Forest or Poinciana). a-petal; b-sepal;c-stigma; d-style; e-filament; f-anther; g-anther of staminoid; h-posterior or standard petal; i-ovary wall; j-ovule.

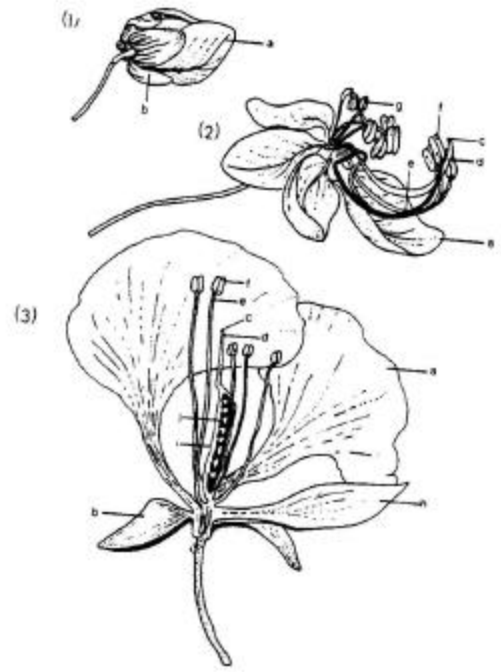


Figure 2-3. Subfamily Mimosoideae.
1. Floret of *Adenanthera pavonina*; 2. in-florescence (globose head) of *Leucaena leucocephala* in longitudinal section showing arrangement of florets on torus; 3. floret of *L. leucocephala* (side view); 4. floret of *L. leucocephala* (top view). a-petal; b-sepal; c-stigma; d-anther; e-filament; f-style; g-ovary

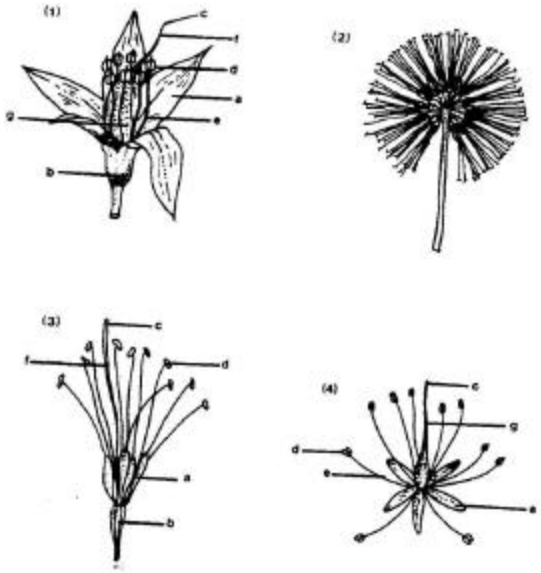


Figure 2-4. Leaves of legumes and associated structures. Leaf shapes: 1. oblong; 2. cuneate; 3. cordate; 4. linear; 5. lanceolate; 6. ovate; 7. oval. Leaf arrangements: 8. bi-pinnate; 9. pinnate; 10. palmate; 11. simple; 12. trifoliate; 13. branch of *Pisum* showing (a) five-branched tendril and (b) stipule; 14. (c) bi-pinnate leaf showing position of pulvinus; 15. *Acacia* seedling showing (d) simple phyllodes, and (e) true compound leaves.

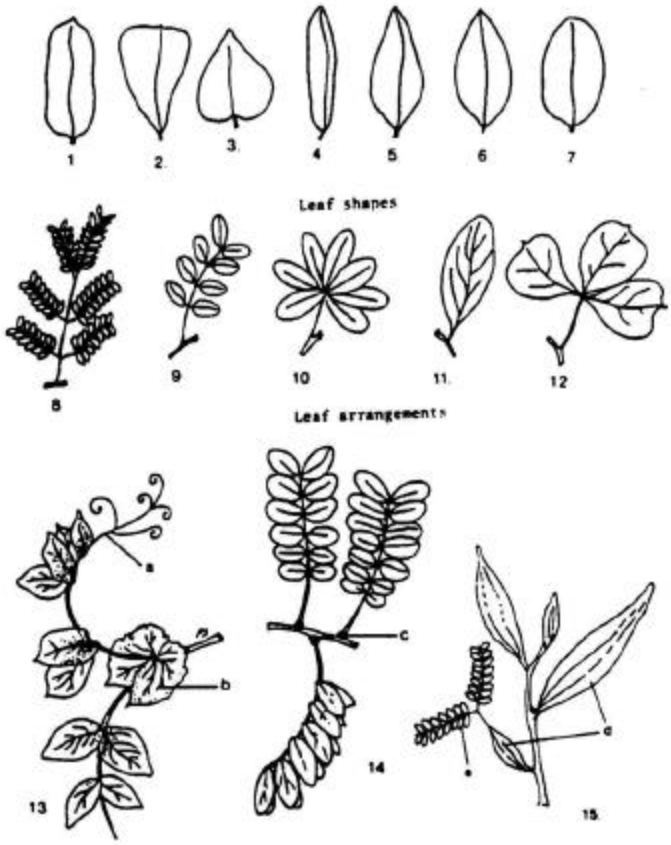
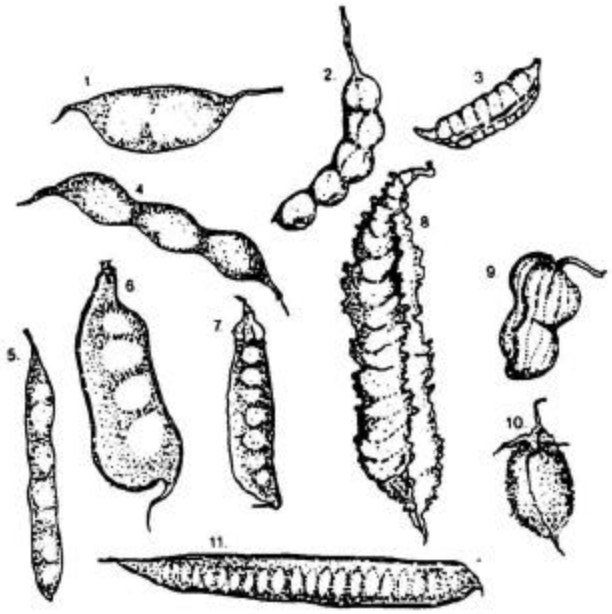


Figure 2-5. Legume pods. 1. *Strongylodon lucidus*; 2. *Tamarindus indica*; 3. *Acacia farnesiana*; 4. *Parkinsonia aculeata*; 5. *Prosopis pallida*; 6. *Lablab purpureus*; 7. *Pisum sativum*; 8. *Psophocarpus tetragonolobus*; 9. *Arachis hypogaea*; 10. *Cicer arietinum*; 11. *Leucaena leucocephala*.



USES

Of the thousands of known legume species, less than 20 are planted extensively today. Those in common use include peanuts (groundnuts), soybeans, peas, lentils, pigeon peas, chickpeas, mungbeans, kidney beans (also known as common or dry beans), cowpeas, alfalfa (lucerne), clovers (*Trifolium* spp.), and vetches. They represent all three subfamilies of the Leguminosae. The Papilionoideae, with a worldwide distribution, are the largest subfamily. They are mostly herbs and include the most important species for human food. The Mimosoideae and Caesalpinoideae are mostly woody trees and shrubs. Many are valuable for lumber, fuelwood, tannins, and animal fodder. **Table 2-2** summarizes the uses of some of the important legumes.

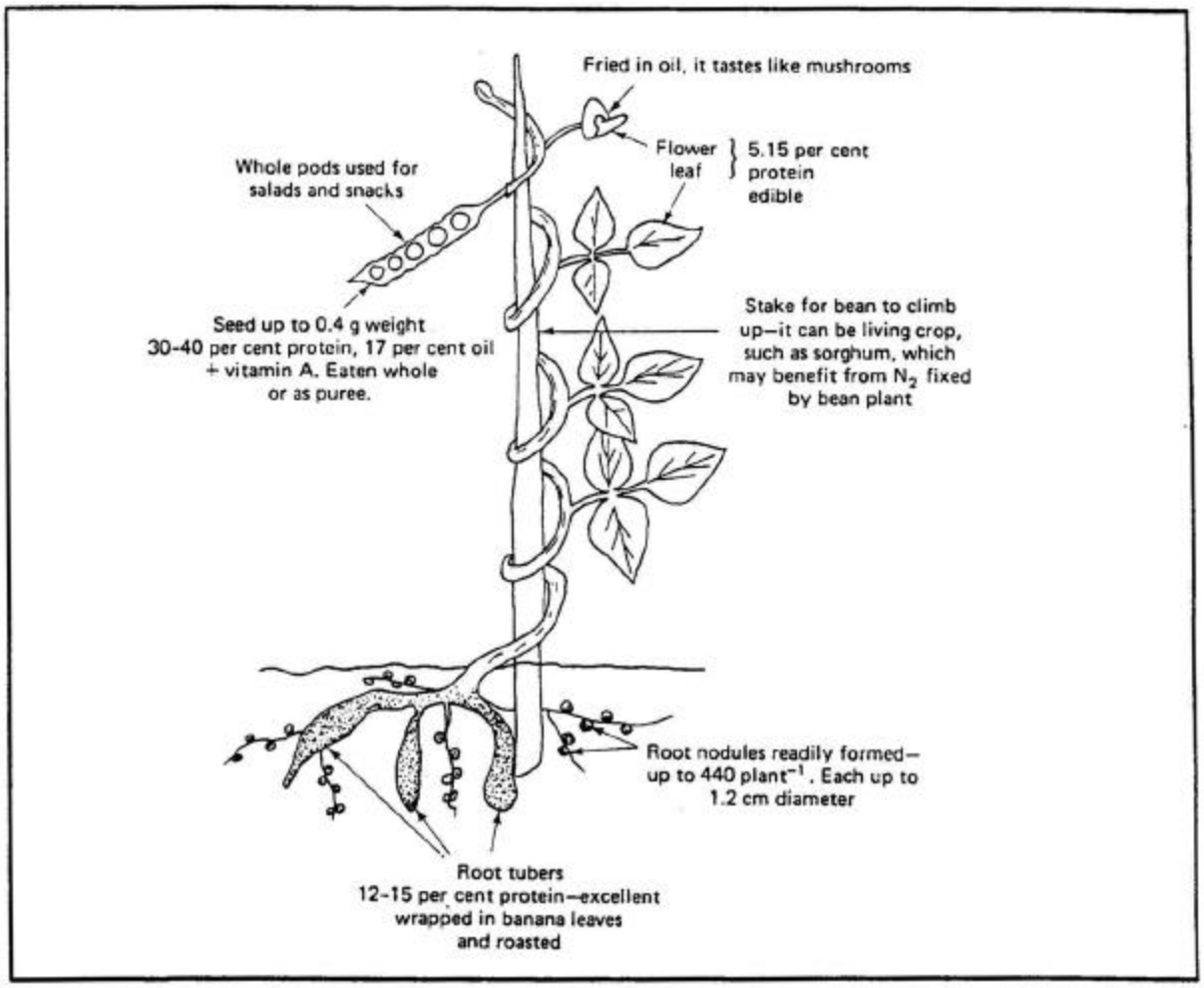


Figure 2-6. The winged bean, just one of many important legumes, is a multi-use plant.

Human Food

Legume seeds (also called pulses or grain legumes) are second only to cereals as a source of human and animal food. When legumes and cereals are eaten together, they provide complete protein nutrition. Nutritionally, legume seeds are two to three times richer in protein than cereal grains. Some legumes, such as soybeans and peanuts, are also rich in oil. Kidney beans and other legumes are a major source of food in Latin America, while lentils, pigeon peas, and chickpeas are important in South Asia. In the Middle East and North Africa, faba beans, lentils, and chickpeas are particularly important. Common food products made from legumes include tofu, peanut butter, and soymilk.

Animal Feed

As standards of human nutrition improve in all countries, there is a corresponding increase in demand for animal products such as milk, butter, eggs, and meat. This demand can only be met by using animal feeds with a high protein content. Among the grain legumes, soybeans are the most extensively used in animal feed.

Forage legumes are commonly provided to animals in grass-legume mixtures. In the temperate regions, clovers, medics, trefoils, and vetches are important. In tropical and subtropical pastures, *Stylosanthes, Pueraria, Lablab, Desmodium,* and other tropical pasture crops are important sources of livestock fodder.

Other Uses

Many species in the Mimosoideae and Caesalpinoideae subfamilies provide valuable timber, dyes, tannins, resins, gums, insecticides, medicines, and fibers. Many provide green manure for crops, such as *Sesbania rostrata* in rice cropping systems and *Gliricidia sepium* and *Leucaena leucocephala* in alley cropping. Many tree legumes have been identified as useful multipurpose species, and these are being introduced through agroforestry, soil restoration, and erosion control programs in many countries.

Table 2-2. Key aspects of selected legume species.

Species (common name)	Habit	Main Uses	Distribution
Subfamily *Mimosoideae*			
Acacia albida	Tree	Fodder, shade	West Africa, Sudan
Acacia auriculiformis	Tree	Shade, ornamental, fuel	Southeast Asia
Acacia farnesiana (cassie, huisache)	Tree	Perfume, tannin, wood, fodder	Australia, India, Java, West Indies
Acacia glauca (syn. *Acacia villosa*)	Tree	Green manure	Indonesia
Acacia koa (koa)	Tree	Fodder, lumber	Hawaii
Acacia lutea	Tree	Fodder	Argentina
Acacia mangium	Tree	Lumber, fuelwood	Southeast Asia
Acacia mearnsii (black wattle)	Tree	Fuelwood, lumber, tannin	South America, East Africa, India
Acacia nilotica (babul, Egyptian mimosa)	Tree	Fodder	Sudan
Acacia pennatula	Tree	Shade coffee, fuel	Central America, Mexico
Acacia senegal (gum arabic, senegal gum)	Tree	Gum arabic	Sudan, Somalia, Senegal, Zambia, Kenya, Ethiopia
Acacia seyal (shittim wood)	Tree	Lumber, fodder	Tropical Africa
Albizia amara	Tree	Browse	India
Albizia falcataria	Tree	shade	Indonesia, Malaysia, Uganda
Albizia lebbek	Tree	Fodder, shade	India, Tropical Africa, West Indies
Albizia sumatrana (syn. *Albizia carbonaria*)	Tree	Shade, green manure	Indonesia, Zaire
Archidendron jiringa (syn. *Pithecellobium lobatum, Pithecellobium jiringa*) (jiring)	Tree	Browse, fodder, food	Indonesia, Malaysia

Species (common name)	Habit	Main Uses	Distribution
Archidendropsis basaltica (syn. *Albizia basaltica*)	Tree	Fodder	Australia
Calliandra calothyrsus	Tree	Fuel, green manure, land reclamation	Indonesia, Philippines
Inga edulis	Tree	Shade for coffee	Colombia, Mexico
Leucaena leucocephala (lamtoro, ipil-ipil, koa haole)	Tree	Green manure, forage fuelwood, land reclamation, paper pulp	South America Asia, Africa
Parkia javanica (petai)	Tree	Food (pods)	Indonesia, Malaysia, Thailand
Pithecellobium dulce	Tree	Fodder, shade	Philippines, Thailand
Prosopis spp. (mesquite)	Tree	Shade, fodder, lumber	Central America, Indonesia, South Africa, USA
Subfamily *Papilionoideae*			
Arachis hypogaea (peanut, groundnut)	Herb	Food	Many tropical countries
Astragalus cicer (cicer milkvetch)	Herb	Forage, erosion control	Canada, USA, Asia, Europe
Cajanus cajan (pigeon pea)	Shrub/tree	Food, green manure, fuelwood	India, Africa, Southeast Asia
Calopogonium mucunoides (calopo, frisolila)	Herb	Erosion control, soil improvement	Java, Malaysia, Sri Lanka, India, Burma
Canavalia ensiformis (jack bean)	Herb	Erosion control, green manure, food	Indonesia, Mexico, Tropical Africa
Cicer arietinum (chickpea, gram, garbanzo)	Herb	Food	Middle East, India, Mexico, Chile, Peru
Crotalaria juncea (sun hemp, Indian hemp)	Herb	Fiber, green manure	India, Pakistan, Bangladesh, brazil
Cyamopsis tetragonoloba (guar, cluster bean)	Herb	Gum, green manure, cover crop, forage	India, Pakistan, USA, Africa

Species (common name)	Habit	Main Uses	Distribution
Dalbergia sissoo (sissoo, shisham)	Tree	Lumber, fodder	India, Pakistan, Nepal
Desmodium spp. (tick clovers)	Herb	Forage	Tropical America, Asia, Africa
Erythrina spp. (coral tree(	Tree	Shade, fodder, green manure, ornamental	All tropical regions
Gliricidia sepium	Tree	Shade, green manure	All tropical regions
Glycine max (soybean)	Herb	Food, fodder	Worldwide
Lens culinaris (lentil, masur dhal)	Herb	Food	Middle East, India, warm temperate regions
Lotus spp. (trefoils)	Herb	Forage	Europe, Middle East, Central Asia, Australia, South America
Lupinus spp. (lupines)	Herb	Forage, green manure, soil improvement	Europe, USA, Mediterranean
Macroptilium spp. (siratro)	Herb	Forage	Central and South America, USA
Macrotyloma uniflorum (horesgram)	Herb	Fodder	India
Medicago spp. (alfalfa, lucerne, medic, burclover)	Herb	Forage	Temperate regions
Melilotus spp. (sweet clover)	Herb	Forage	Worldwide
Pachyrhizus erosus (yam bean, jicama, sen kuang)	Herb	Food	Mexico, Southeast Asia, China
Phaseolus coccineus (scarlet runner bean)	Herb	Food	Europe, Central America
Phaseolus lunatus (lima bean, butter bean)	Herb	Food	Indonesia, Burma, USA, Central America, Africa
Phaseolus vulgaris (bean, common bean)	Herb	Food	Most temperate and subtropical regions
Pisum sativum (common	Herb	Food, fodder	Most temperate and

Species (common name)	Habit	Main Uses	Distribution
or garden pea)			subtropical regions
Psophocarpus tetragonolobus (winged bean)	Herb	Food	Indonesia, New Guinea, Burma, Thailand, Malaysia
Pueraria phaseoloides (kudzu, puero)	Herb	Forage, erosion control	Southeast Asia
Sesbania grandiflora	Tree	Green manure, food	Indonesia, Philippines, Malaysia, India
Sesbania rostrata	Tree	Green manure, food	West Africa, Philippines, Tropical Americas, Australia, Southeast Asia
Stylosanthes spp. (stylo)	Herb	Forage	Tropical Americas, Australia, Southeast Asia
Trifolium spp. (clovers)	Herb	Forage	USA, Canada, Australia, Mediterranean region
Vicia faba (broadbean, faba bean)	Herb	Food	USA, Canada, Middle East, South America
Vigna mungo (urdbean, black gram)	Herb	Food	India, Pakistan
Vigna radiata (gram, mungbean)	Herb	Food	India, China, Indonesia, Thailand, USA
Vigna subterranea (syn. *voandzeia subterranea*) (bambara groundnut)	Herb	Food	Africa, Southeast Asia
Vigna umbellata (rice bean)	Herb	Food	Africa, Asia, USA
Vigna unguiculata (kacang, cowpea)	Herb	Food	Africa, Asia, USA
Subfamily *Caesalpinoideae* (none of the species listed fixes nitrogen)			
Bauhinia spp.	Tree/shrub	Forage, fodder, ornamental	southeast Asia, Tropical Africa

Species (common name)	Habit	Main Uses	Distribution
Cassia alata	Herb	Medicine, tannin	West Africa
Cassia senna	Herb	Cosmetic	North Africa, Egypt
Ceratonia siliqua (carob, locust)	Tree	Food, gum	Mediterranean region
Senna occidentalis (syn. *Cassia occidentalis*)	Herb	Medicine	Indonesia, Africa, Sri Lanka
Tamarindus indicus (tamarind)	Tree	Food, medicine, wood	Southeast Asia, India, Africa

REVIEW AND DISCUSSION

- List the legumes commonly used in agriculture in your country. Can you identify them by their scientific names and assign them to their subfamilies?
- What are the forage legumes in your country? Which animals feed on these forages?
- Which tree legumes are used in land reclamation and agroforestry in your country?
- Which grain legumes are used to produce edible oils in your country?
- Identify as many legumes as you can from plant specimen. Find out their scientific and common names. Are these legumes introduced or native to your country?

SUGGESTED LESSON PLAN FOR MODULE 2.

TIME: One hour +

OBJECTIVES:

Knowing what plants are legumes, i.e., how to identify them. Knowing the many uses of legumes. Knowing that most legumes form nodules.

MATERIALS:

Samples of native legumes for display -nodulated and non-nodulated

Training aids for Module 2

STEPS:

1. Display key concepts and other appropriate training aids. Gather legume samples yourself or, if appropriate, have the group go out and gather samples.

2. Explain some of the identifying characteristics of legumes, i.e., shape of flowers and. leaves, presence of pods, and nodules on roots. This step is done very well in the field where the whole plant can be examined in place.

3. Again, use questions regarding the types of legumes the audience is familiar with, their uses, etc. This leads into the lecture which can be quite short.

KEY CONCEPTS

Legumes are among the three largest families of flowering plants and have a long history of use in agriculture.

Not all legumes are nodulated.

Legumes belong to the family Leguminosae which consists of four subfamilies, the Papilionoideae, Caesalpinoideae, Mimosoideae, and Swartzioideae.

Examining the reproductive structure is the most dependable way to identify and recognize the legume subfamilies.

Legumes have multiple uses.

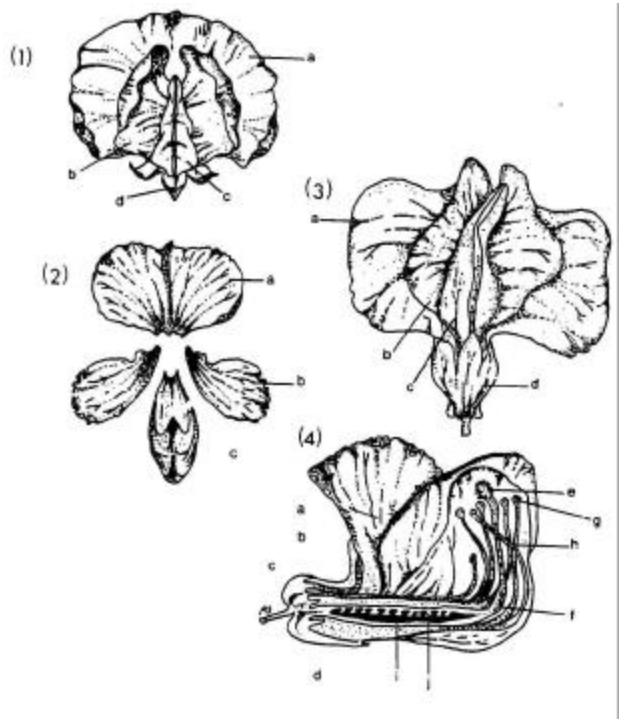


Figure 2-1. SubfamilyPapilionoideae. 1. front view of flower of *Pisum sativum* (pea); 2. petals of *P. sativum*; 3. flower of *Psophocarpus tetragonolobus* (winged bean); 4. flower of *P. tetragonolobus* in longitudinal section. a-posterior or standard petal; b-lateral petal; c-keel petals (carina); d-sepals; e-stigma; f-style; g-anther; h-filament; i-ovary wall; j-ovule.

Figure 2-2. Subfamily Caesalpinoideae.
1. bud of *Cassia* sp.; 2. flower of *Cassia* sp.; and 3. longitudinal section through flower of *Delonix regia* (Flame of the Forest or Poinciana). a-petal; b-sepal;c-stigma; d-style; e-filament; f-anther; g-anther of staminoid; h-posterior or standard petal; i-ovary wall; j-ovule.

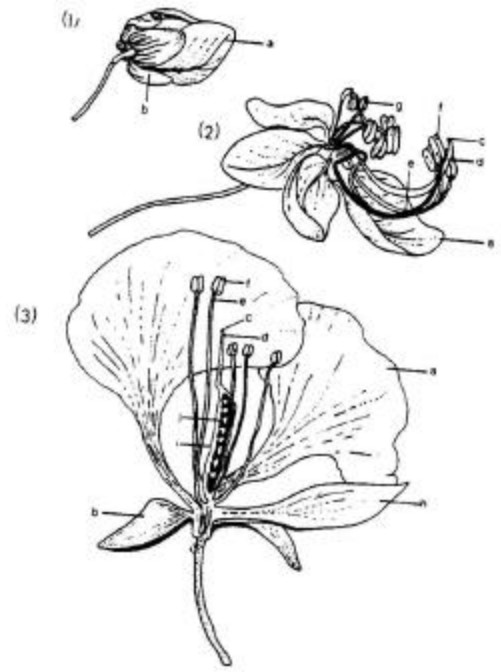


Figure 2-3. Subfamily Mimosoideae.
1. Floret of *Adenanthera pavonina*; 2. in-florescence (globose head) of *Leucaena leucocephala* in longitudinal section showing arrangement of florets on torus; 3. floret of *L. leucocephala* (side view); 4. floret of *L. leucocephala* (top view). a-petal; b-sepal; c-stigma; d-anther; e-filament; f-style; g-ovary

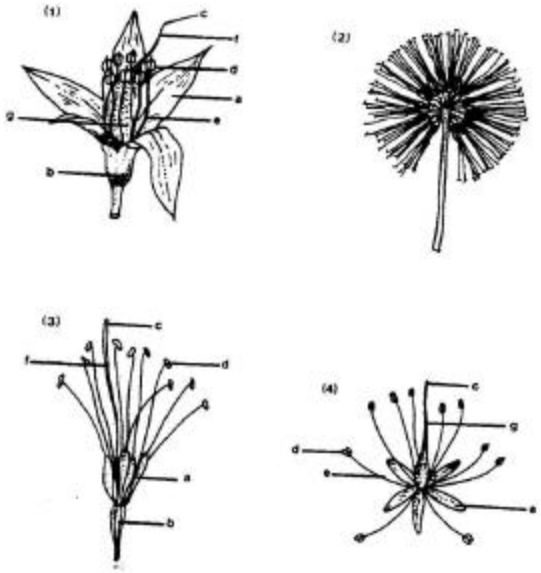


Figure 2-4. Leaves of legumes and associated structures. Leaf shapes: 1. oblong; 2. cuneate; 3. cordate; 4. linear; 5. lanceolate; 6. ovate; 7. oval. Leaf arrangements: 8. bi-pinnate; 9. pinnate; 10. palmate; 11. simple; 12. trifoliate; 13. branch of *Pisum* showing (a) five-branched tendril and (b) stipule; 14. (c) bi-pinnate leaf showing position of pulvinus; 15. *Acacia* seedling showing (d) simple phyllodes, and (e) true compound leaves.

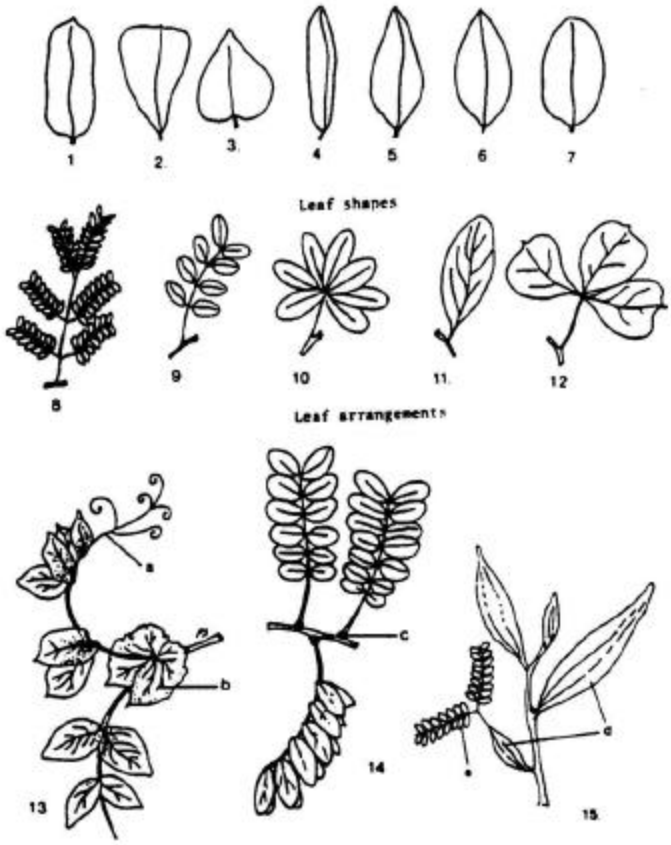
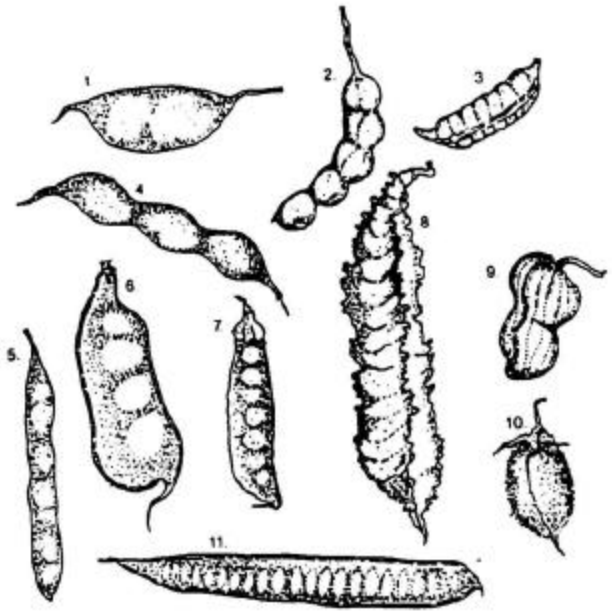


Figure 2-5. Legume pods. 1. *Strongylodon lucidus*; 2. *Tamarindus indica*; 3. *Acacia farnesiana*; 4. *Parkinsonia aculeata*; 5. *Prosopis pallida*; 6. *Lablab purpureus*; 7. *Pisum sativum*; 8. *Psophocarpus tetragonolobus*; 9. *Arachis hypogaea*; 10. *Cicer arietinum*; 11. *Leucaena leucocephala*.



MODULE NUMBER 3

INTRODUCTION TO RHIZOBIA

SUMMARY

This module introduces the general role of microorganisms in the soil, and specifically the rhizobia. Rhizobia are special bacteria that can live in the soil or in nodules formed on the roots of legumes. In root nodules, they form a symbiotic association with the legume, obtaining nutrients from the plant and producing nitrogen in a process called biological nitrogen fixation, or BNF. The rhizobia are broadly classified as fast- or slow-growing based on their growth on laboratory media. Rhizobia are further classified according to their compatibility with particular legume species. Farmers can stimulate BNF by applying the correct rhizobia to their legume crops, a process called inoculation. The module describes the diversity of rhizobia and the selection of superior strains, as well as plant and environmental factors that affect rhizobia in the soil.

KEY CONCEPTS

- Soil microorganisms play many important roles.
- Rhizobia are special soil microorganisms that can form a symbiotic relationship with legumes resulting in biological nitrogen fixation, or BNF.
- Rhizobia are classified according to the legume species that they nodulate — a concept known as "cross-inoculation" groups.
- To achieve effective BNF, legumes must be inoculated with the correct rhizobia.
- Superior strains of rhizobia can be selected as inoculants.
- Plant and environmental factors affect native and introduced rhizobia in the soil.
- Native rhizobia can affect the results of inoculation.

SOIL MICROORGANISMS

The soil contains many types of microorganisms—microscopic forms of animal life such as bacteria, actinomycetes, fungi, and algae. Soil microorganisms are important because they affect the soil's physical, chemical, and biological properties. For example, the process of decay, breakdown, and disappearance of dead animal and plant material is largely due to the action of many different types of microorganisms. The conversion of animal and plant waste into nutritionally rich compost also results from the action of microorganisms.

RHIZOBIA ARE SPECIAL SOIL BACTERIA

Amongst the soil bacteria there is a unique group called **rhizobia** that have a beneficial effect on the growth of legumes. Rhizobia are remarkable bacteria because they can live either in the soil or within the root nodules of **host** legumes.

When legume seeds germinate in the soil, the root hairs come in contact with rhizobia. If the rhizobia and the legume are compatible, a complex process begins during which the rhizobia enter the plant's root hairs. Close to the point of entry, the plant develops a root nodule. **Module 4** describes this **infection** of legume roots by rhizobia in more detail.

Once the relationship between plant and rhizobia is established, the plant supplies the rhizobia with energy from photosynthesis and the rhizobia **fix** atmospheric nitrogen in the nodule, converting it into a form that the plant can use. Both the plant and the rhizobia benefit from such a relationship called a **symbiosis**. The rhizobia living in the plant's root nodules are called **symbionts**.

The complex process by which the rhizobia produce nitrogen for the legume is called **biological nitrogen fixation**, or **BNF**. Only rhizobia that are specifically compatible with a particular species of legume can stimulate the formation of root nodules, a process called **nodulation**. This process has great economic benefit for legume production. As a result, rhizobial **inoculants** are produced commercially in many countries. Inoculants contain rhizobia isolated from plant nodules and grown (cultured) artificially in the laboratory. **Modules 4** and **5** describe the symbiosis and rhizobia production and use in more detail.

Growth Characteristics in the Laboratory

In the laboratory, rhizobia are grown on a special medium called yeast-mannitol agar (YMA). They are grouped in two main genera—the **fast-growing** *Rhizobium* species and the **slow-growing** *Bradyrhizobium* species. When cultured on YMA, the *Rhizobium* species produce visible growth in two to three days. They produce an acid growth reaction, which can be detected by adding a pH indicator, bromthymol blue (BTB), to the medium. Rhizobia isolated from pea, bean, clover, alfalfa, chickpea, and leucaena are all fast-growers. The *Bradyrhizobium* species take six to eight days to produce visible growth on YMA and produce an alkaline reaction. The soybean and cowpea rhizobia are slow-growers.

Physical Characteristics

Rhizobia grown in the laboratory are shaped like short rods, as seen under the microscope. They measure 0.5 to 0.9 ìm wide and 1.2 to 3.0 ìm long. Like most living things, they need a supply of air (oxygen) to live. They can move using special thread-like structures called flagella.

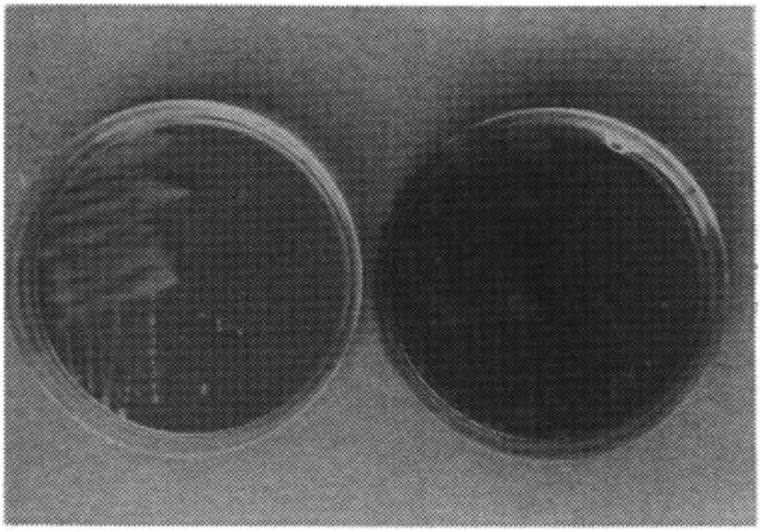


Figure 3-1. Rhizobia grown on YMA media have a gummy or slimy appearance.

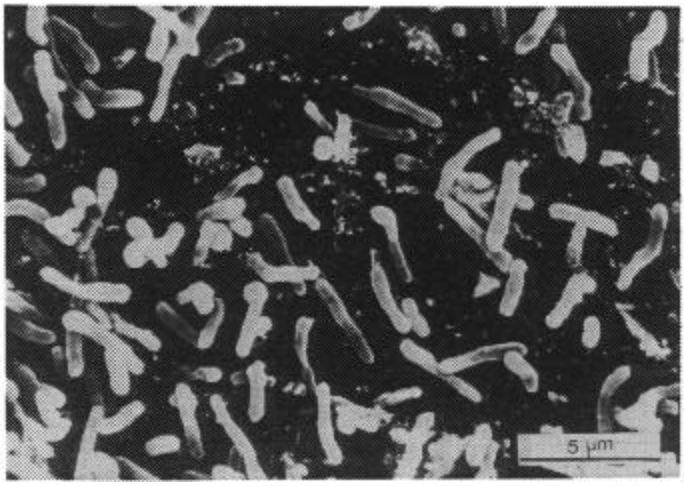


Figure 3-2. Rod shaped rhizobia in the nodule of cowpea (*Vigna unguiculata*).

CROSS-INOCULATION GROUPS: CAN ONE RHIZOBIAL STRAIN NODULATE ALL LEGUMES?

Not all rhizobia nodulate all legumes. Rather, particular rhizobia form a symbiosis with particular legumes or groups of legumes. The symbiosis between rhizobia and legumes appears to be precisely matched, although in some cases a certain level of mismatching is tolerated. To obtain the full benefits of BNF, it is extremely important to provide farmers with the correct rhizobia for their legume crop.

Scientists have studied this matching system for many important food, forage, and tree

legumes. They have categorized rhizobia and their legume partners into **cross-inoculation groups**. Each of these groups consists of all the legume species that will develop nodules when inoculated with rhizobia obtained from any other member of the same group.

Although this matching system is not perfect, it is a valuable guide for farmers and extension workers. **Table 3-1** gives some of the most important cross-inoculation groups. It shows that some rhizobia can nodulate in a broad range of legumes, while others are very specific. Likewise, some legume hosts require very specific types of rhizobia, while others can form symbiotic associations with rhizobia from many other hosts. Such legumes are considered **promiscuous**.

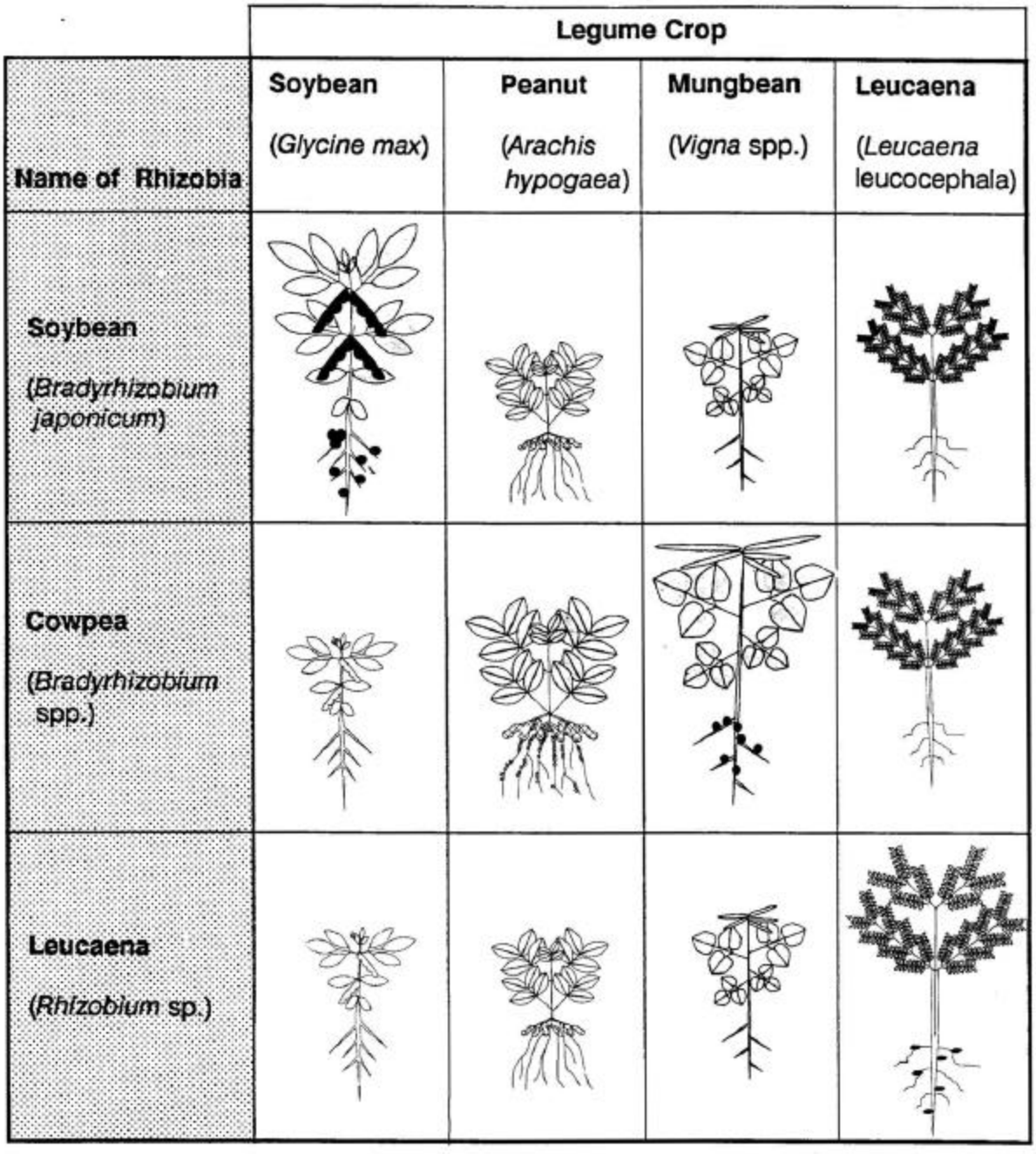
Table 3-1 shows that we should not inoculate soybean with pea rhizobia, for instance, or leucaena with soybean rhizobia. What happens if we do? In some cases, no nodules will form. This means that there will be no nitrogen fixation to benefit the legume. In other cases, nodules may form, but they will be **ineffective**, meaning they will not fix nitrogen.

When legume and rhizobia are well matched, the **effective** nodules are deep red inside. This is clearly visible when you cut them open. Nodules that are green or white inside will be ineffective. **Module 4** will discuss nodule color in more detail.

Table 3-1. Rhizobia and the cross-inoculation groups of legumes they nodulate.

Names of rhizobia	Legume cross-inoculation groups
Pea Rhizobia (*Rhizobium leguminosarum* bv. viceae)	**Pea Group** peas (*Pisum* spp.); vetches (*Vicia* spp.); lentils (*Lens culinaris*); faba bean *Vicia faba*)
Bean Rhizobia (*R.I.* bv. phaseoli)	**Bean group** beans (*Phaseolus vulgaris*); scarit runner bean (*P. coccineus*)
Clover Rhizobia (*R.I.* bv. trifolii)	**Clover group** clovers (*Trifolium* spp.)
Alfalfa Rhizobia (*R. meliloti*)	**Alfalfa group** alfalfa (*Medicago* spp.); sweet clovers (*Melilotus* spp.); fenugreek (*Trigonella* spp.)
Chickpea Rhizobia (*Rhizobium* sp.)	**Chickpea group** Chickpea (*Cicer arietinum*)
Soybean Rhizobia (*Bradyrhizobium japonicum*)	**Soybean Group** soybeans (*Glycine max*)
Leucaena Rhizobia (*Rhizobium* sp.)	**Leucaena group** leucaenas (*Leucaena leucocephala; L. shannoni; L. lanceolata; L. pulverulenta*); *Sesbania grandiflora; Calliandra callothyrsus; Gliricidia sepium; Acacia farnesiana; Prosopis* spp.
Cowpea Rhizobia (*Bradyrhizobium* spp.)	**Cowpea group** pigeon pea (*Cajanus cajan*); peanut (*Arachis hypogaea*); cowpea, mungbean, black gram, rice bean (*vigna* spp.); lima bean (*Phaseolus lunatus*); *Acacia mearnsii; A. mangium; Albizia* spp.; *Enterlobium* spp., *Desmodium* spp., *Stylosanthes* spp., Kacang bogor (*Voandzeia subterranea*), *Centrosema* sp., winged bean (*Psophocarpus tetragonolobus*), hyacinth bean (*Lablab purpureus*), siratro (*Macroptilium atropurpureum*), guar bean (*Cyamopsis tetragonoloba*), calopo (*Calopogonium mucunoides*), puero (*Pueraria phaseoloides*)

Figure 3-3. Examples of using the cross-inoculation groups for selecting the proper rhizobial inoculant for the legume host. The proper combination of rhizobia and legume will result in the best nodulation and most nitrogen fixation. We see that using soybean rhizobia with soybean forms an effective symbiosis, while soybean rhizobia on leucaena does not. Using information from Table 3-1 shows that cowpea rhizobia nodulates both mungbean and peanut.



STRAINS: IS ONE RHIZOBIUM AS GOOD AS ANOTHER?

Even among rhizobia that can nodulate the same plant, there are many different, genetically distinct, strains. Some fix nitrogen better—more efficiently—than others, resulting in superior plant growth. Some also compete better with rhizobia that are already in the soil. This means that they can enter the plant's root hairs more efficiently, resulting in faster nodule formation. Inoculant producers need to select superior strains for their product.

Strains of rhizobia from separate nodules are kept in culture collections at research laboratories and at factories where rhizobial inoculant is produced. Scientists test these strains on legume plants, and the best are selected for use in inoculant pro-duction. Selecting highly effective rhizobia is an important aspect of BNF research.

In some tests, plants are grown in pots under carefully controlled conditions with only one strain of rhizobia and no other source of nitrogen. Superior strains will fix the most nitrogen, and plants inoculated with these will be the greenest, healthiest and largest.

For example, **Figure 3-4** shows that strain Tal 22 produced superior growth in rice bean (*Vigna umbellata*). Rice bean plants inoculated with TAL 22 grew better, as indicated by shoot dry weight, that plants inoculated with nine other rhizobial strains. The uninoculated control plants grew very poorly since they had no source of nitrogen. Plants inoculated with TAL 22 grew almost as well as plants receiving a high amount of nitrogen fertilizer (the N control).

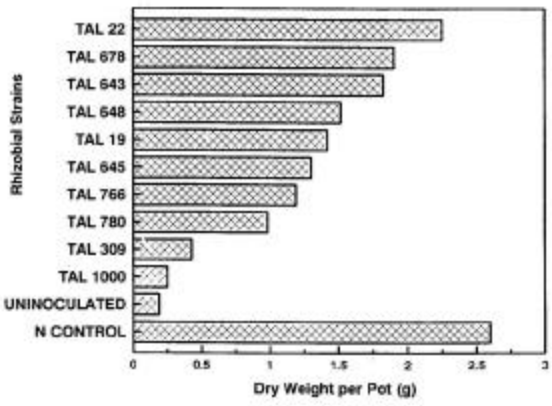


Figure 3-4. Effect of inoculation with strains of rhizobia on shoot dry weights of rice bean (*Vigna umbellata*) grown in the greenhouse at NifTAL, Maui, Hawaii.

Scientists have found superior rhizobial strains for each of the commercially important legume crops. They have also identified strains that compete successfully with other rhizobia to form nodules. Research now focuses on finding even better strains for major crop species, effective strains for less well-known species such as the tree legumes, and strains that survive and induce nodulation under difficult conditions such as high temperatures or drought.

NATIVE RHIZOBIA

When rhizobia live in the soil, they are called saprophytes. Many soils contain rhizobia that live on soil organic matter, without legume partners. These are called **native** rhizobia, while those that farmers add as inoculants are called **introduced** rhizobia. The population of native rhizobia in any soil can be very diverse, including several species, and many distinct strains within each species. Numbers can range from zero to more than a million rhizobia per gram (g) of soil. Several factors affect the number of rhizobia in the soil. These include vegetation, cropping history, and environmental and soil conditions.

VEGETATION AND CROPPING HISTORY

In many agricultural soils, the presence of vegetation—whether legumes or non-legumes—appears to encourage large numbers of rhizobia. Rhizobia are found in especially large numbers in the region close to the roots of plants, known as the **rhizosphere**.

While non-legume vegetation can encourage native rhizobia, the largest numbers of rhizobia are generally found in areas with wild or cultivated legumes. The particular species of native rhizobia present in an area depends on the species of legume growing in the soil. For example, if a farmer grows peanuts (groundnuts) in a field for many years, you may expect to find a large number of native rhizobia that can form a symbiosis with peanut. Remembering the cross-inoculation groups (**Table 3-1**), these peanut rhizobia can also be expected to stimulate nodulation in mungbean, but not in soybean.

Table 3-2 shows how cropping systems affect the number of native rhizobia in the soil. The rhizobia in this table nodulate members of the cowpea cross-inoculation group, which includes species such as cowpea, peanut, mungbean, and hyacinth bean. Where rice was cropped in rotation with hyacinth bean (*Dolichos lablab*), the numbers of rhizobia were high. By contrast, there were only few rhizobia in soils where legumes had not been cropped for many years.

When a new crop is introduced to an area, it may take some time to build up the soil rhizobial population. A survey in Lampung and Sumatera Barat, Indonesia, provides an example (P.W. Singleton, Saroso S., B. Hilton, and N. Boonkerd, unpublished data). Among fields where soybeans had grown for only one year, none had more than 100 soybean rhizobia per gram of soil. After two or more years of soybean cropping, 30% of the fields tested still had fewer than 100 soybean rhizobia per gram soil. Where numbers of native rhizobia are this low, farmers are advised to apply rhizobial inoculant to benefit fully from BNF.

Table 3-2. Effect of cropping systems on cowpea rhizobia numbers at six sites in Karnataka State, India.

Cropping System	No. of Years	Number of Rhizobia per g soil

Rice/Legume	>6	5,000
Rice/Legume	>6	230,327
Rice/Legume	>6	197,782
Rice	10	6
Sugar	10	11
Sugar	21	10

Source: Unpublished data from P.W. Singleton and N.G. Hegde. Rice/legume system is rice crop (*Oryza sativa*) followed by lablab (*Dolichos lablab*).

Table 3-3. The effect of soybean cultivation on the number of soybean rhizobia in the soils of Lampung and Sumatera Barat.

Number Rhizobia per g Soil	Years of Soybean Cultivation	
	1	>2
	- - - - - - - - - - % soils - - - - - - - - - -	
0 - 100	100	30
> 100	0	70

ENVIRONMENT AND SOIL FACTORS

Besides vegetation and cropping history, the population of rhizobia in the soil is influenced by the environment. One of the most important factors is rainfall. Areas with adequate rainfall often have large numbers of native rhizobia because the rhizobia themselves survive well in moist soils and also because more rainfall usually means that there are more legumes and other plants. Rhizobia prefer soils that are moist but not waterlogged.

Other conditions, such as soil temperature and acidity (pH), are also important. Rhizobia prefer a soil temperature of 25° to 30°C and a pH of 6.0 to 6.8. Rhizobia are sensitive to low pH (acid soils). **Table 3-4** shows that liming soils in Sumatera had a beneficial effect on the numbers of cowpea rhizobia.

Table 3-4. The relationship between soil pH and the number of cowpea rhizobia in the Sumateran soils.

Number Rhizobia	Soil pH

per gram soil	less than 5.4	greater than 5.4
	- - - - - - - - - - % Fields - - - - - - - - - -	
0 - 100	44	0
> 100	56	100

(P.W. Singleton, Saroso S., B. Hilton, and N. Boonkerd, unpublished data).

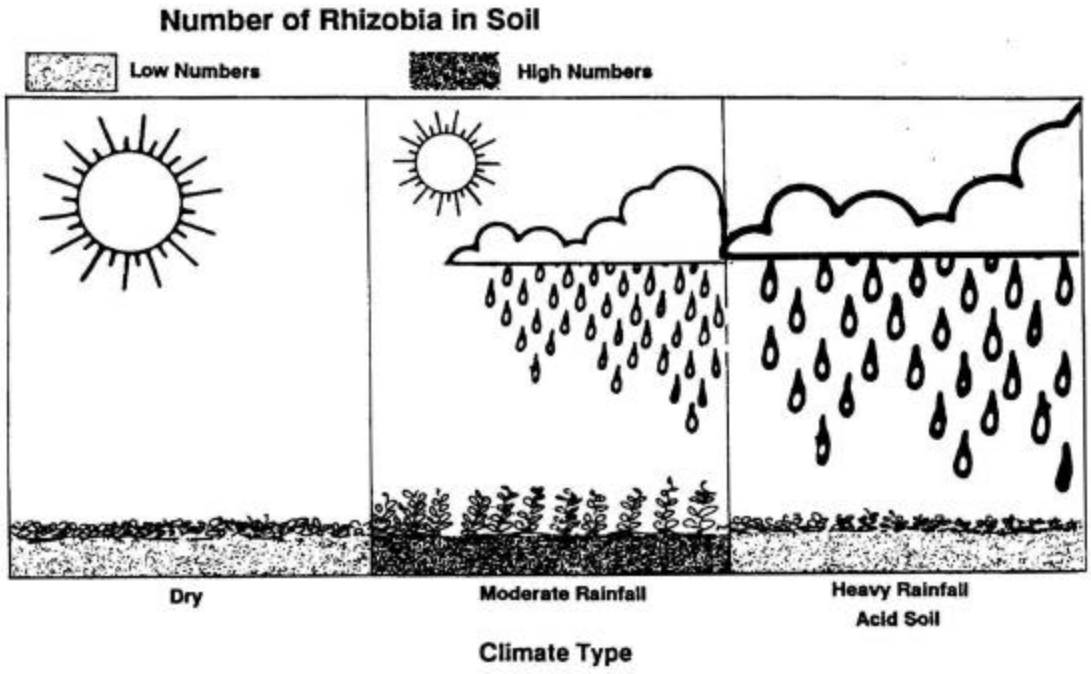


Figure 3-5. The effect of climate on rhizobial populations.

THE IMPORTANCE OF NATIVE RHIZOBIA

It is important to consider the numbers and types of native rhizobia in the soil because they can affect the results of inoculating legume seeds with introduced rhizobia. Remember that native rhizobial populations are diverse, containing effective and ineffective strains. These native rhizobia can affect the results of inoculation in two ways. If there are already many native rhizobia in the soil that can nodulate a legume crop and induce BNF, then inoculating seed may not produce any further benefits. For example, if a farmer has grown peanuts for some years, there may be many native rhizobia in the soil that are effective on peanut. Adding more rhizobia in an inoculant may not make any difference.

On the other hand, native rhizobia may **compete** with introduced rhizobia to form nodules on legume plants. In this case, the nodules on the legume are formed by the native rhizobia and not by the rhizobia introduced in the inoculant. If the native rhizobia are not as effective at fixing nitrogen as the introduced strains, then plants will not get the maximum benefit from BNF.

PERSISTENCE OF INTRODUCED RHIZOBIA

If farmers inoculate one legume crop, will they have to inoculate again the next time they plant the same legume? In other words, will introduced rhizobia **persist**, or continue to live in the soil until the next crop? Not necessarily. Once rhizobia are introduced to the soil, they will be affected by the same factors that affect native rhizobia—vegetation, soil moisture, pH, and temperature. Without doing a thorough soil analysis, it is difficult to predict whether effective inoculant strains are present from previous crops. It is safer to inoculate again.

Again, the study from Sumatera, Indonesia, provides an example (P.W. Singleton, Saroso S., B. Hilton, and N. Boonkerd, unpublished data). When farmers inoculated their soybean crops, even for several years, and then did not plant soybean for one year, nearly half (47%) of their fields had less than 100 soybean rhizobia per gram of soil. When rhizobial populations are this low, farmers should continue to use inoculant to get the maximum benefit from BNF. Because the potential benefits are worth much more than the price of the inoculant, farmers should always be encouraged to inoculate their legumes even if they have inoculated previous crops.

Table 3-5. Years since inoculation of soybean and the number of soybean rhizobia in Sumateran soils.

Number Rhizobia per gram soil	Years from Inoculation	
	1	**>2**
	- - - - - - - - - - % Fields - - - - - - - - - -	
0 - 100	47	43
> 100	53	57

Source: Unpublished data from Singleton, Saroso, Hilton, Boonkerd.
Data are the proportion of fields with rhizobia in the range of 0-100/g soil or greater than 100/g soil.

REVIEW AND DISCUSSION

- A farmer has grown soybean in the same field for three years using rhizobial inoculant. This year, she could not get the inoculant, so she planted her crop without inoculating the seed. Six weeks later, you (the extension agent) visit the farmer and she tells you that she is happy with her soybean. You dig up a few soybean plants and find them to be well nodulated. The nodules are red inside and the leaves look healthy and green. How is it that the soybeans were nodulated without inoculation?
- Later in the discussion, the same farmer tells you that she will not inoculate future crops because the plants looked good without inoculation. What will you advise? What if she plans to expand her soybean production to other fields?
- You visit another farmer as he is preparing to plant soybeans. He tells you that he grew peanuts in previous years, and that he has inoculated his soybean seeds with the same inoculant he normally uses on peanut. Should he plant his inoculated soybean seeds? What alternatives could you suggest?
- A group of nursery specialists grow casuarina seedlings for distribution to farmers. (Although these trees fix nitrogen, they are not legumes.) They make their own inoculant by picking and crushing root nodules of mature casuarinas. They have seen you inoculating legume seeds with inexpensive commercial inoculants and would like to try these on their seedlings. What would you advise?

SUGGESTED LESSON PLAN FOR MODULE 3

TIME: One hour +

OBJECTIVES:

Knowing what rhizobia are and how they interact with legumes. Understanding the importance of matching rhizobia and a particular legume using the cross-inoculation method.

MATERIALS:

Demonstrations D3/1 and D3/2

Training Aids for Module 3

STEPS:

1. Display key concepts and other appropriate training aids.

2. Decide on how much of this lecture is appropriate for your audience. Be creative in deciding how to explain microorganisms and their possibilities, i.e., the effect of yeast in bread and beer. Be careful, however, not to mislead the group about what rhizobia are.

3. Selectively present from the module that information which will be useful. You might bring in the idea of native rhizobia by now examining the nodules on any of the legume samples collected in the last module. The color of the nodule is important to discuss.

KEY CONCEPTS

The importance of soil microorganisms.

Rhizobia are special soil bacteria that are responsible for BNF with legumes.

Rhizobia are classified by the legumes they nodulate.

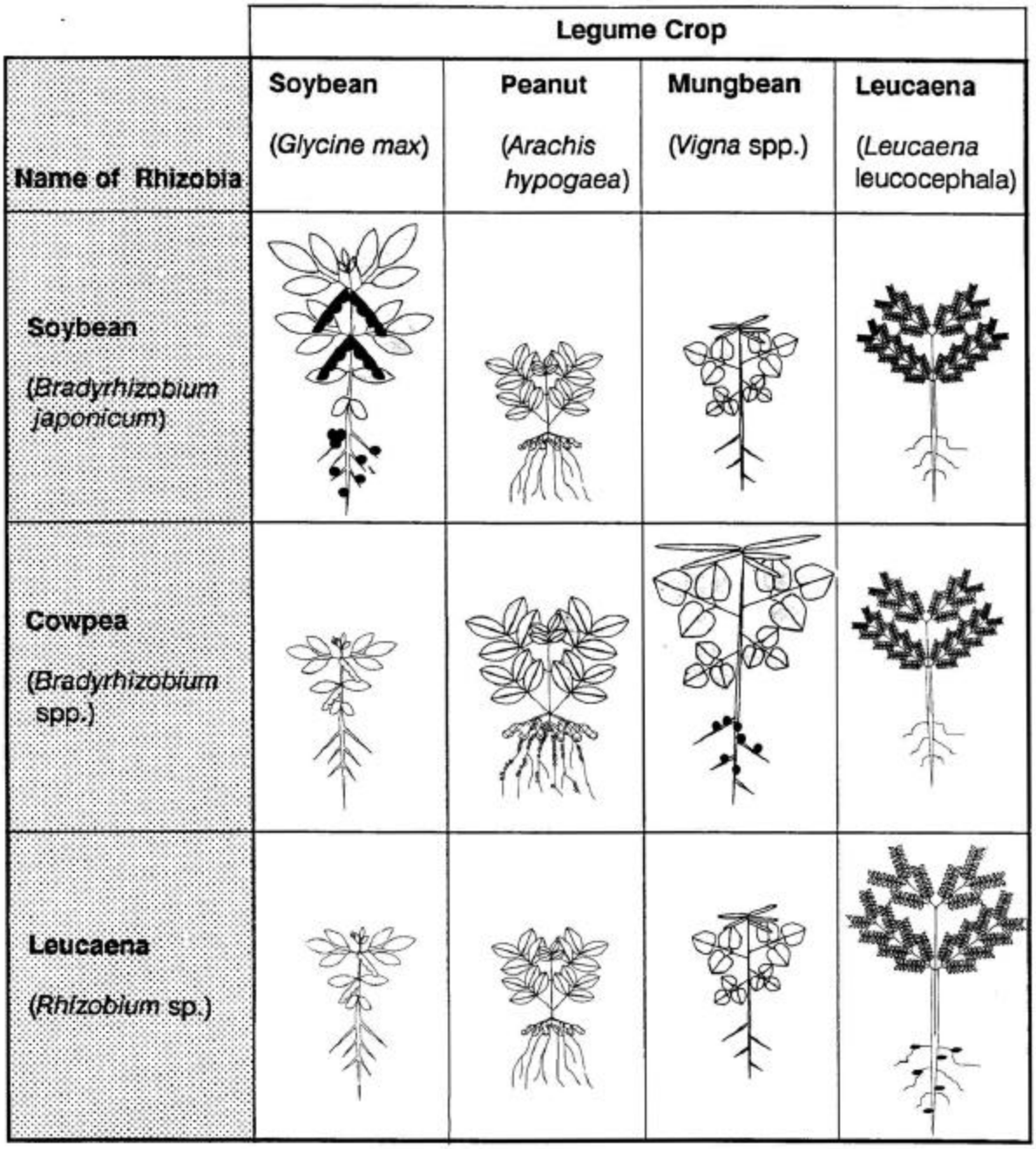
Rhizobial inoculants must be properly matched with the legume.

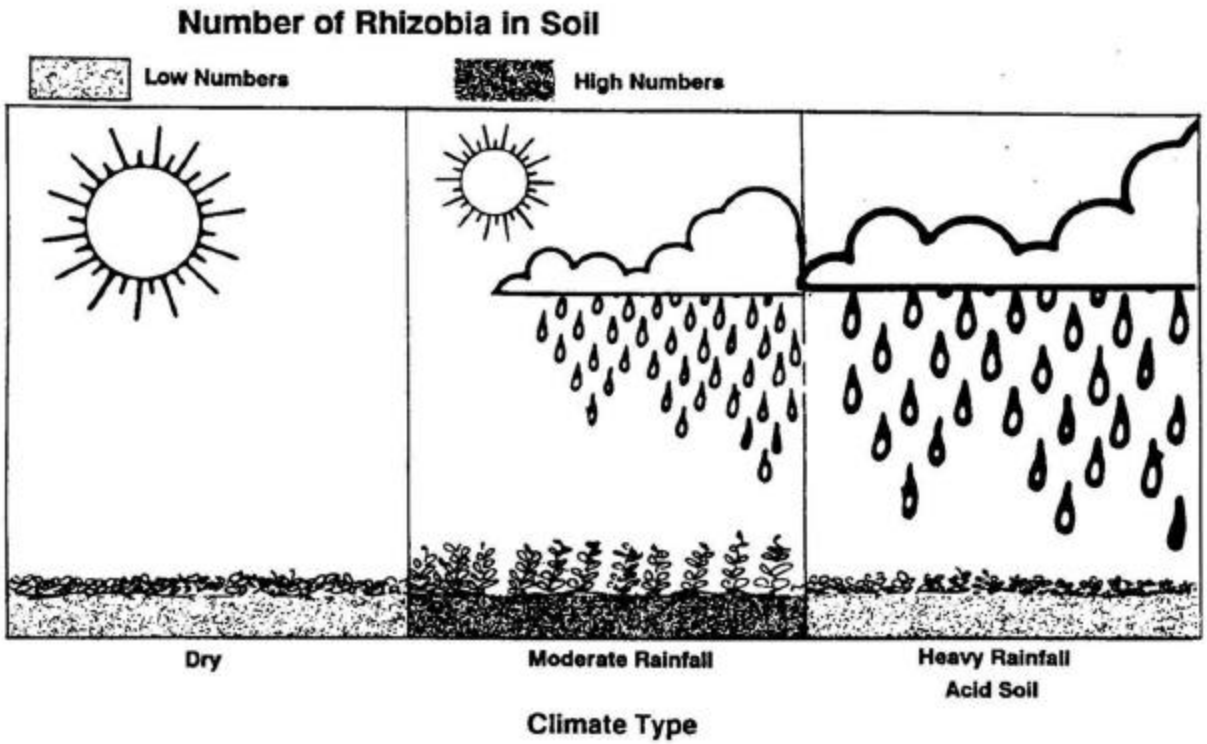
"Superior" strains of rhizobia can be selected for inoculants.

The soil and environment affecting native and introduced rhizobia in the soil.

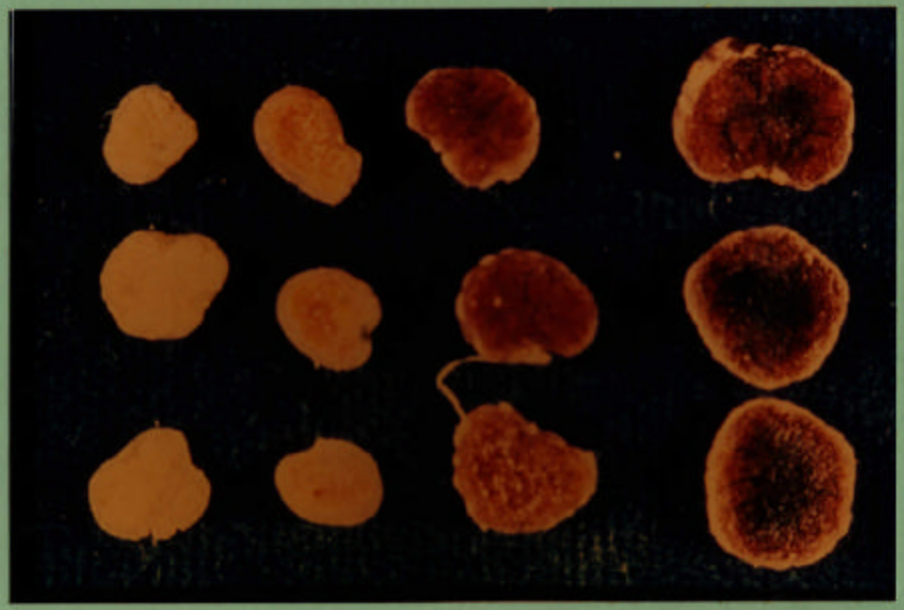
The importance of native rhizobia.

Figure 3-3. Examples of using the cross-inoculation groups for selecting the proper rhizobial inoculant for the legume host. The proper combination of rhizobia and legume will result in the best nodulation and most nitrogen fixation. We see that using soybean rhizobia with soybean forms an effective symbiosis, while soybean rhizobia on leucaena does not. Using information from Table 3-1 shows that cowpea rhizobia nodulates both mungbean and peanut.




Number of Rhizobia in Soil
Low Numbers
High Numbers
Dry
Moderate Rainfall
Heavy Rainfall
Acid Soil
Climate Type

MODULE 3



MODULE NUMBER 4

THE LEGUME-RHIZOBIA SYMBIOSIS

SUMMARY

The legume-rhizobia symbiosis consists of several stages: 1) infection of legume roots by rhizobia; 2) nodule development; 3) nodule function; and 4) nodule senescence. This module discusses how BNF works and follows the rate of nitrogen produced by BNF. Several production systems are described that use legumes to take advantage of BNF.

KEY CONCEPTS

- The BNF symbiosis consists of complex processes of infection of roots by rhizobia, nodule development, nodule function, and nodule senescence.
- The amount of nitrogen fixed by a legume depends on several factors, most importantly the level of nitrogen already available in the soil: BNF is most active when soil nitrogen is minimal.
- Legumes differ greatly in the amount of nitrogen they leave in the field for subsequent crops. The concepts of harvest index, nitrogen harvest index, and percent nitrogen from BNF are useful for estimating nitrogen inputs from legumes and benefits to the cropping system.
- In addition to producing valuable food and animal feed, legumes are beneficial as rotational crops, green manure, cover crops, forage, and fuelwood.

STAGES OF THE LEGUME-RHIZOBIA SYMBIOSIS

Infection

Whether native to the site or introduced through inoculation, rhizobia must be able to survive in the soil until they infect the roots of a plant. Generally, these microorganisms survive well in soil, but their numbers can be reduced by acidity, drought, high temperatures, or other stress conditions. If the rhizobia are compatible with a given legume species, they will multiply in the root zone and attach to the **root hairs** of the plants. The root hairs are fine structures on the roots that absorb water and nutrients. After the rhizobia attach, they use the root hair as an entry point into the plant (**Figure 4-1**). In some cases, rhizobia may also enter through "cracks" or breaks in the root surface where lateral roots emerge.

The rhizobia enter the plant by forming an **infection tunnel**, or **infection thread**, through several cell layers to the site where a nodule will develop. Once inside the plant, the rhizobia are protected to some extent from stresses in the outside environment.

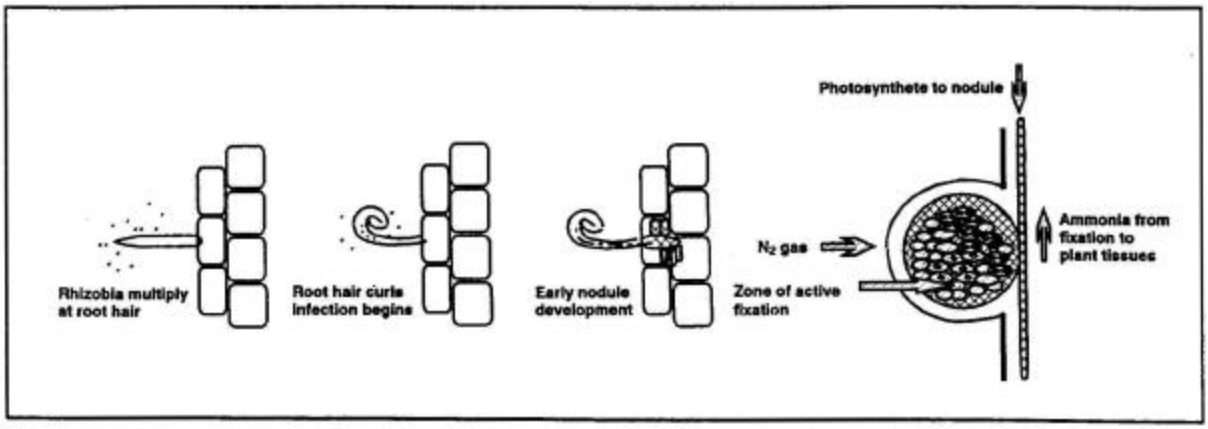


Figure 4-1. Stages of infection, nodule development and nodule formation.

Nodule Development

The rhizobia end their journey at the site of the future nodule. There, special plant tissues develop around them. These include connective (vascular) tissues through which the plant feed sugars to the rhizobia and the rhizobia feed nitrogen back to the plant. As these and other tissues develop, the root begins to swell and the nodule becomes visible. In the field, nodules are visible within 21 to 28 days from emergence of the plant. The time from planting to the appearance of nodules varies depending on plant growth and availability of mineral nitrogen in the soil.

Nodules differ in shape, size, color, texture, and location. Their shape and location depend largely on the host legume. **Figure 4-2** shows some of the common nodule shapes, including spherical, finger-like, and fan-shaped. A few species belonging to the genera *Sesbania, Aeschynomene,* and *Neptunia* also form nodules on the plant skins.

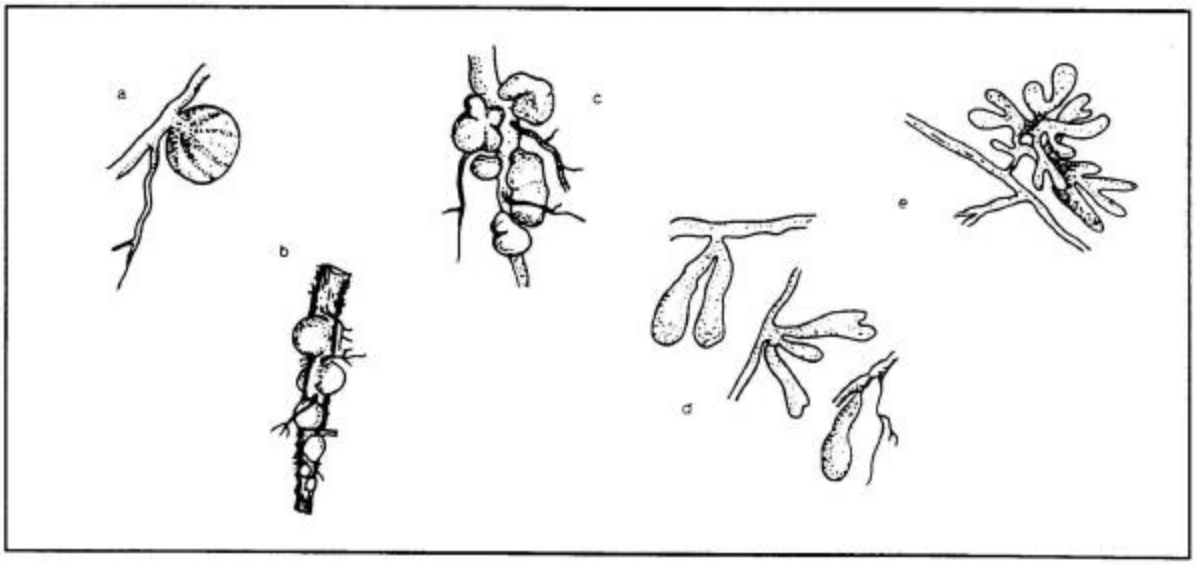


Figure 4-2. Some representative shapes of leguminous nodules. Spherical: a. globose and streaked, e.g., *Glycine max*, *Calopogonium*, and *Vigna radiata* and *Psophocarpus*. Finger-like forms: d. elongate and lobed, e.g., *Leucaena* and *Mimosa*. Fanshaped: e. coralloid, e.g., *Crotalaria* and *Calliandra*.

Nodule Function

Within the developing nodule, the rhizobia become swollen. At this stage they are called **bacteroids**. In a cycle depicted in **Figure 4-3**, Nitrogen gas (N_2) from the soil atmosphere reaches the bacteroids through pores in the nodule. The bacteroids produce the enzyme **nitrogenase**, which they use to convert N_2 to NH_3 (**ammonia**). The ammonia attaches to a compound provided by the plant, forming amino acids. These amino acids move out of the nodule to other parts of the plant where they undergo further changes. They are mainly used to produce proteins.

The bacteroids need large amounts of energy to support their nitrogen-fixing activity. The plant provides energy as sugars, produced through photosynthesis. It is estimated that the legume-rhizobia symbiosis requires about 10 kg of carbohydrates (sugars) for each kg of N_2 fixed. Clearly, the plant must be healthy to supply enough energy to support BNF. In addition to sunlight, it must have enough water and other nutrients.

As discussed in **Module 3**, legume plants will generally produce nodules in response to several different strains of rhizobia, but not all these strains will be fully **effective** in fixing nitrogen. Some will be poor nitrogen fixers, many mediocre, and a few will be very good. Some strains may even induce nodulation but will not fix nitrogen at all. Inoculant obtained from a reputable source should contain only rhizobial strains that are highly effective nitrogen fixers.

Nodules produced by effective rhizobia are usually large. They tend to be located in the

upper portion of the root system on the primary and lateral roots. In annual legumes, the number and size of nodules reach a peak about the time of flowering. Nitrogen fixation is also at its peak at this time. By contrast, nodules produced by ineffective rhizobia tend to be small. They are often quite numerous, scattered throughout the root system.

Young, healthy nodules that are providing nitrogen to the plant are often pink or red inside. As they age, they may contain white, green, and red areas, all within a single nodule. Ineffective nodules tend to be white or light green inside throughout the growing season, and they are often smooth textured. The NifTAL/FAO manual, *Legume inoculants and their use* (1984), gives examples of different nodule shapes and colors.

Nodule Senescence

Eventually nodules age and decay. Their life span is largely determined by four factors: the physiological condition of the legume, the moisture content of the soil, the presence of any parasites, and the strain of rhizobia forming the nodule.

As an annual legume approaches maturity, it fills developing seeds with nutrients and storage compounds. As the plant puts more energy into seed production, the nitrogen-fixing activity of the bacteroids decreases. Eventually the nodules stop functioning and disintegrate, releasing bacteroids into the soil. Given favorable conditions, these rhizobia may survive and infect new plants during the next cropping season. However, in intensive agricultural systems it is usually necessary to add rhizobial inoculant with every crop.

Plants may shed their nodules early if affected by severe drought. Forage legumes also shed nodules after heavy grazing,but these species can often produce new nodules. Finally, some crops may be susceptible to parasites, such as weevil larvae, that feed on root nodules.

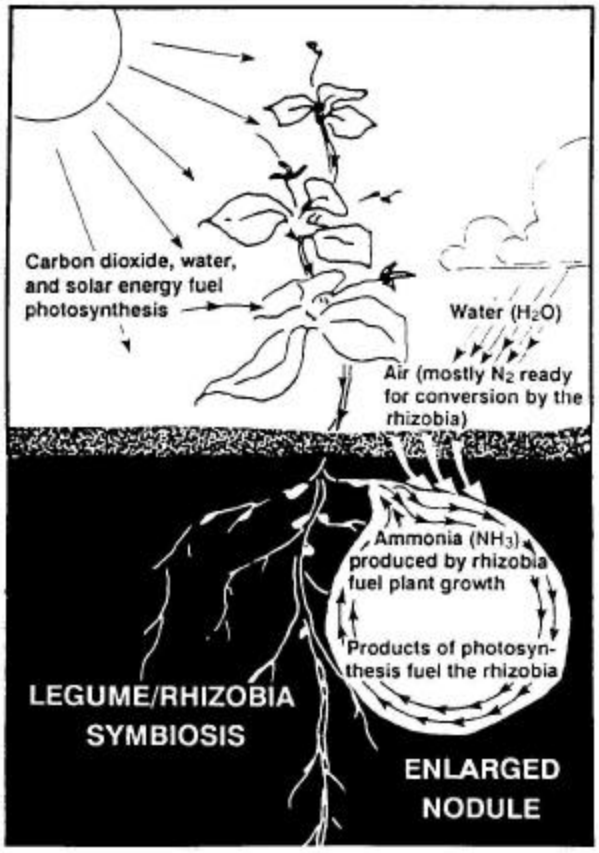


Figure 4-3. The legume-rhizobia symbiosis.

FACTORS AFFECTING NITROGEN FIXATION

Legumes are diverse in growth habit, size, and length of growing season. They also differ in the amounts of nitrogen they can fix, even under ideal conditions. **Figure 4-4** gives some estimates of nitrogen fixation by different legume species. The NifTAL/FAO manual gives a more extensive list of legume species and the amounts of nitrogen they fix.

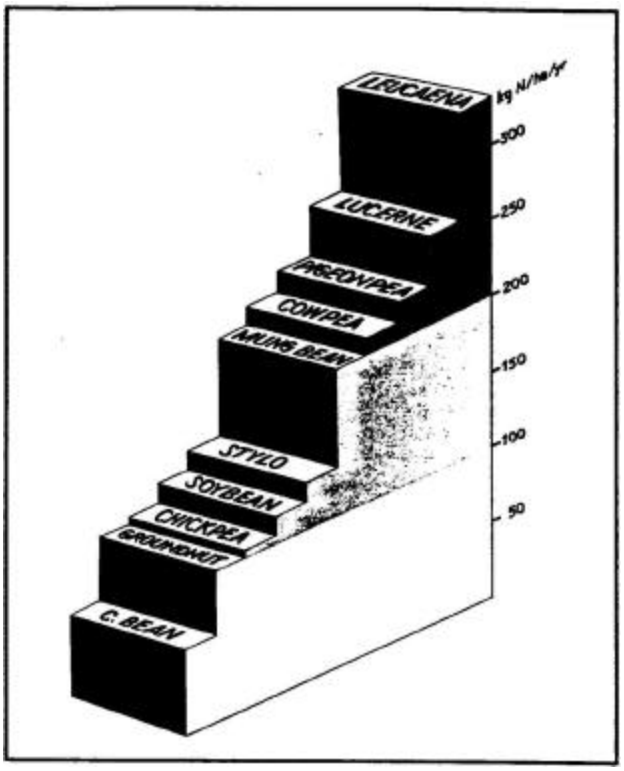


Figure 4-4. Amounts of nitrogen fixed by various legumes. From *Inoculants and Their Use,* 1984, UNFAO/NifTAL

Legumes can obtain nitrogen from three sources—soil nitrogen, native rhizobia, and rhizobia introduced as inoculants. In most cases, legumes will obtain some of their N from the soil, even if they fix high amounts of N_2. As long as other plant health factors (water, pests, nutrients, etc.) are not limiting, the amount of nitrogen fixed by legume plant depends on the abundance and longevity of the root nodules, the Effectiveness of BNF within the nodules, and the level of available soil nitrogen. As a general principle, nitrogen fixation goes up as soil nitrogen goes down, and vice versa. Given high levels of nitrogen in the soil, plants may not form nodules at all, or they may reduce or cease nitrogen-fixing activity in the nodules already formed.

Table 4-1 illustrates the effect of nitrogen fertilizer on nodule formation. In this example, increasing levels of nitrogen fertilizer reduced the abundance of nodules in both soybeans and common beans. Comparing uninoculated and inoculated crops, we see that the native rhizobia in this field induced nodulation in the common beans but not in the soybeans. Nitrogen fertilization reduced nodulation with both native and introduced rhizobia.

Table 4-1. Effect of fertilizer nitrogen on nodule dry weight of soybean and

common bean at the end of flowering.

	Soybean		Common Bean	
N applied	**Uninoc.**	**Inoc.**	**Uninoc.**	**Inoc.**
kg/ha	- - - - - - - - - - - - - - - kg nodules/ha - - - - - - - - - - - - - - -			
3	0	86	46	69
40	0	67	27	57
300	0	33	5	5

From t. George, Ph.D. thesis, University of Hawaii, 1988.
Data are dry weight of nodules

Table 4-2 illustrates the effect of nitrogen fertilizer on the amount of nitrogen fixed by soybeans. Increasing levels of nitrogen fertilizer reduced nitrogen fixation. Given a choice, the plants used nitrogen from fertilizer (mostly in the form of NO_3) rather than obtaining nitrogen from BNF. These results were obtained experimentally by adding nitrogen fertilizer in measured doses, but the principle would be the same in situations where soil nitrogen is already high. The amount of soil N at the time of planting is determined by previous crops, additions of fertilizers and manures, the amount of soil organic matter, and the environment (especially moisture and temperature). Again as a general principle, the less nitrogen there is in the soil, the more legume plants will rely on BNF.

Table 4-2. The effect of nitrogen fertilization on nitrogen fixation by soybeans at the end of flowering and at maturity.

N applied (kg/ha)	End of flowering	Maturity
	- - - - - - - - - - kg N/ha from BNF - - - - - - - - - -	
9	37	168
120	25	109
900	20	41

Source: T. George, 1988. Ph.D. thesis, University of Hawaii.

ROLE OF NITROGEN FIXATION IN THE PRODUCTION SYSTEM

It is commonly assumed that legumes enrich the soil with nitrogen; however, even legumes that are fixing nitrogen, may still take up substantial amounts of nitrogen from the soil. Increases and decreases in soil nitrogen depend on the type of legume grown, the management system, and the amount of nitrogen already in the soil.

Both grain legumes (soybean, mungbean, cowpea, peanut) and forage legumes (alfalfa, clover) take nitrogen from the soil, but grain legumes tend to take more because most of their nitrogen is transferred to the seed, which is then harvested and removed from the

system. Forage legumes are more likely to increase the nitrogen content of the soil, enhancing yields of companion or subsequent crops. Many forage legumes grow for longer periods and develop more extensive root systems than grain legumes. Their roots and nodules contain considerable amounts of nitrogen that remain in the soil even after the plants are harvested.

In pastures, most nitrogen fixed by forage legumes passes through the grazing animals and returns to the soil in urine and feces, where it can potentially benefit a companion grass crop. Up to 80% of the nitrogen fixed by legumes and returned to the soil is in the form of animal waste, and 70% of this is in urine.

Without animals, nitrogen returns to the production system when stems, leaves, roots, and nodules are incorporated in the soil and allowed to decompose. Microbes in the soil mineralize the organic nitrogen, converting it to a form that can be used by subsequent crops. Because not all nitrogen is mineralized at once, the legumes may provide residual nitrogen over a two- to three-year period.

Two concepts are useful in evaluating the contribution of legumes to the nitrogen fertility of soil—the harvest index and the nitrogen harvest index. These are calculated as follows:

$$\text{Harvest Index} = \frac{\text{Weight of grain (or other economic yield)}}{\text{Weight of shoot and grain}}$$

$$\text{Nitrogen Harvest Index} = \frac{\text{Weight of nitrogen in harvested grain}}{\text{Weight of nitrogen in shoot and grain}}$$

Table 4-3. An example of the calculations required to estimate the contribution of BNF to soil nitrogen levels. The total yield (grain plus stover) from a soybean crop at Kuiaha was 8283 kg/ha and the grain yield was 4424 kg/ha, giving a harvest index of 0.53. This means that 53% of the total yield was harvested and removed from the system.

Site	Grain (kg/ha)	Stover (kg/ha)	Total Nitrogen (kg/ha)	Grain Nitrogen (kg/ha)	Nitrogen Produced by BNF		Nitrogen Taken from Soil	
					(%)	(kg/ha)	(%)	(kg/ha)
Kuiaha	4424	3859	317	278	82	260	18	57
Haleakala	3066	3381	246	212	71	175	29	71

Source: T. George et al., 1988. *Agronomy Journal*. 80:563-67.

Nitrogen yields were calculated by analyzing the nitrogen component of crop samples. The remaining 47% of the yield is stover (3,859 kg) with about 1% N content, or 39 kg/ha. Stover is often burned or fed to animals but, in this case, if the stover were returned to the field, the net loss of nitrogen from the system would be reduced from 57 to 18 kg/ha.

Although few data are available on the quantity of roots left in the soil by legumes, it has been estimated for soybean to be about 50% of the weight of harvested grain. At Kuiaha, this would be about 2212 kg/ha. The roots contain about 1% nitrogen, which means that about 22 kg/ha of nitrogen would be returned to the soil from the roots of this soybean crop.

The nitrogen harvest index is a measure of how much nitrogen is recovered out of the total nitrogen contained in a crop. Common estimates are 70% and higher for soybean and wheat and somewhat lower for maize (P.B. Cregan and P. van Berkum, 1984. *Theoretical and Applied Genetics*. 67:97–111). At Kuiaha, out of 317 kg/ha of nitrogen in the grain and stover, 278 kg/ha were harvested in the grain, giving a nitrogen harvest index of 0.87, or 87%. Since this is higher than the proportion of nitrogen derived from BNF (82%), it means that there was a net removal of nitrogen from the soil. Had the nitrogen harvest index and the percent of nitrogen derived from BNF been the same, there would have been no change in soil nitrogen. Had the percentage of nitrogen derived from BNF been higher, there would have been a net addition of nitrogen to the soil. These calculations help us understand the nitrogen balances in cropping systems and estimate the inputs that may be required to maintain soil nitrogen levels.

PRODUCTION SYSTEMS THAT USE BNF

The previous examples examined the legume/rhizobia symbiosis in annual grain legume cropping systems. Other systems also take advantage of the BNF activity of legumes and contribute to the sustainability of cropping systems.

Legumes in Crop Rotations

Legumes have been used in crop rotations for centuries. Usually their main purpose is to produce high-protein forage for livestock. An additional, valuable benefit is the nitrogen supplied to subsequent crops. **Table 4-4** gives some examples of nitrogen fixed by legume crops and the effects on productivity of subsequent cereal crops. As mentioned previously, the forage legumes (alfalfa, clover, sweet clover) usually provide more nitrogen to subsequent crops than the grain legumes (soybean, common bean). Data are taken from the fifth rotation of legume crop followed by cereal crop.

In these rotations, cereal yields largely depended on the amount of nitrogen the legume added through BNF and the amount that was removed when the legumes were harvested. For the alfalfa and clovers, the net addition was considerable, but the soybean and common bean harvest removed more nitrogen than the plants had fixed. Yields of the subsequent cereal crops reflected this loss of soil nitrogen.

Table 4-4. Nitrogen fixed by leguminous crops and their influence on a following cereal crop (barley or rye).

		Nitrogen harvested		
	Total N fixed by legume	**Legume crop**	**Cereal crop**	**Yield of cereal grain***
	- kg/ha -			
Alfalfa	505	335	74	2920
Clover	290	140	57	2440
Sweet Clover	300	190	57	2370
Soybean	180	197	32	1480
Common Bean	80	115	28	1330
Cereal every year			25	1090

From E.W. Russell. 1973. Soil conditions and plant growth. 10th edition, Longman, London.
*Yield of cereal crop is after five cycles of the crop system. One cycle is a legume crop followed by a cereal crop.

In another trial, the legume *Lupinus angustifolius* (lupin) was grown in rotation with wheat. The lupin fixed 252 kg/ha of nitrogen, which was 96% of the total nitrogen contained in the plants. Only 86 kg/ha of nitrogen was removed when the lupin was harvested, giving a nitrogen harvest index of 33%. The net contribution to soil nitrogen was therefore 166 kg/ha. **Table 4-5** shows the benefit to the subsequent wheat crop compared to benefits obtained from nitrogen fertilizer. At this site, a farmer would have had to apply between 60 and 80 kg/ha of nitrogen fertilizer to produce a wheat yield equal to the level obtained when the previous crop was lupin and no nitrogen was added.

Table 4-5. Grain yields of wheat following wheat or lupin with six rate of fertilizer N.

	- - - - - - - - - - Nitrogen Fertilizer Applied (kg/ha) - - - - - - - - - -					
Previous Crop	**0**	**20**	**40**	**60**	**80**	**100**
Wheat	2020	2430	2930	2900	3400	3000
Lupin	3280	3440	3550	3770	3690	3480

Source: D.F. Herridge, 1982. *In* J.M. Vincent (ed.) Nitrogen Fixation in Legumes. Sydney, Academic Press.

Legumes as Green Manure

When the entire legume is returned to the soil (the harvest index is zero), there is maximum benefit to the following crop. This management practice replenishes soil organic matter as well as nitrogen. A legume used in this way is called a **green manure**. This practice require labor to plant the legume, harvest it, and dig it back into the soil without obtaining any products from the harvest, and there must be sufficient time between primary crops. However, the benefits can be considerable, as demonstrated in **Table 4-6**. In this example, rice yields were substantially higher following a green-manure crop of *Sesbania rostrata* than with nitrogen fertilizer applied at a rate of 60 kg/ha. On balance, green manuring as a management practice must be evaluated in each location. In some cropping systems, the practice may not be economical even though it enhances the nitrogen fertility of the system.

Table 4-6. Influence of inoculated *Sesbania rostrata* green manure on the yield and total nitrogen content of a subsequent rice crop.

	Rice Dry Matter Yield		Rice Nitrogen Content		Rice Nitrogen Yield	
	Grain (kg/ha)	Straw (kg/ha)	Grain (%)	Straw (%)	Grain (kg/ha)	Straw (kg/ha)
Green manure	5960	7720	1.80	0.84	107.3	74.4
Nitrogen (go kg/ha as NH_4SO_4	3810	4840	1.27	0.49	48.3	23.8
Untreated	2120	2760	1.14	0.58	24/2	16.0

Source: Rinaudo et al., 1982. *In* P.H. Graham and S.C. Harris (eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture. Cali, Colombia: CIAT.

Legumes as Cover Crops

In some areas, the need to promote soil fertility and to protect the soil from erosion caused by heavy rainfall has led to the introduction of fast-growing legume cover crops between rows of plantation cash crops. The cover crops protect for the soil when the plantation crops are young, and soil fertility is increased by mineralization of leaf fall from the legume.

Such cover crops are used in the production of rubber, oil palm, and coconut. Legumes planted in these systems must be shade tolerant. They must also be able to compete with the accompanying cash crop for nutrients and water, but without being so competitive that they inhibit growth of the cash crop. In Malaysia, the legumes *Pueraria phaseoloides, Calopogonium mucunoides* and *Centrosema pubescens* were grown in a mixture under rubber trees and compared with a mixture of grasses and with natural cover. **Table 4-7** shows that the legume mixture provided high levels of nitrogen and other nutrients as leaf litter, which became available to the plantation crop.

Table 4-7. Amount of nutrients in litter of different cover plants at 24 months after

planting in a rubber plantation.

Cover plants	Dry weight of litter	N	P	K	Mg
		- - - - - - - - - - kg/ha - - - - - - - - - -			
Leguminous mixture	6038	140	11	31	19
Grass mixture	6140	63	9	31	16
Natural cover	5383	64	6	42	17

Source: C.Y. Kuan, 1982. *In* P.H. Graham and S.C. Harris (eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture, Cali, Colombia: CIAT.

Legumes in Mixed Pastures

Forage legumes are important for pasture improvement and livestock production. Animal scientists have long recognized that mixed grass-legume forages are superior to nitrogen-fertilized grass in terms of animal performance. Legumes can contribute to pasture production by providing high-protein forage, especially during the dry season when grass quality is poor. Several documented cases show marked increases in pasture productivity and animal weight gain after introducing a nitrogen-fixing forage legume. Examples are the introduction of *Trifolium* sp., *Medicago* sp., *Centrosema pubescens*, *Calopogonium mucunoides*, and *Stylosanthes guianensis* in Australia, *Desmodium intortum* in Uganda, *Pueraria phaseoloides* in Puerto Rico, and *Neonotonia wightii* in Brazil (P.J. Skerman, 1977. *Tropical forage legumes*. Rome: FAO).

REVIEW AND DISCUSSION

- Discuss the nitrogen balance in the main legume crops grown in your area. Are these crops likely to add nitrogen to the soil for subsequent crops? Do they fix most of their own nitrogen? What factors determine the amount of nitrogen they fix?
- The section on the Role of Nitrogen Fixation in the Production System discussed a trial in Hawaii in which returning soybean stover to the soil, instead of burning it or feeding it to animals, would add about 39 kg/ha of nitrogen to the soil. Given the current price of nitrogen fertilizer, would it be worthwhile for most farmers to spend the time and effort to return the stover to the soil? If not, at what price of fertilizer might this practice be worthwhile?

SUGGESTED LESSON PLAN FOR MODULE 4

TIME: 2 hours +

OBJECTIVES:

Understanding how the legume-rhizobia symbiosis works. Knowing how to calculate the amount of nitrogen gained. Knowing what cropping systems can be used with legumes.

MATERIALS:

Demonstrations D4/1 and D4/2

Training Aids for Module 4

STEPS:

1. Decide if you will be able to do an effective demonstration. This could be combined in a field demonstration if you have an appropriate setting and advance preparation time.

1. Display key concepts and appropriate training aids. Begin with review questions about rhizobia and legumes. This should give you a basis to begin the lecture on this topic.

2. Review the module resource materials and determine what you will cover in depth. Again, the knowledge level of the audience will be the main issue for determining what you will cover.

3. Using the demonstrations, offer information appropriate to the participants skilllevel.

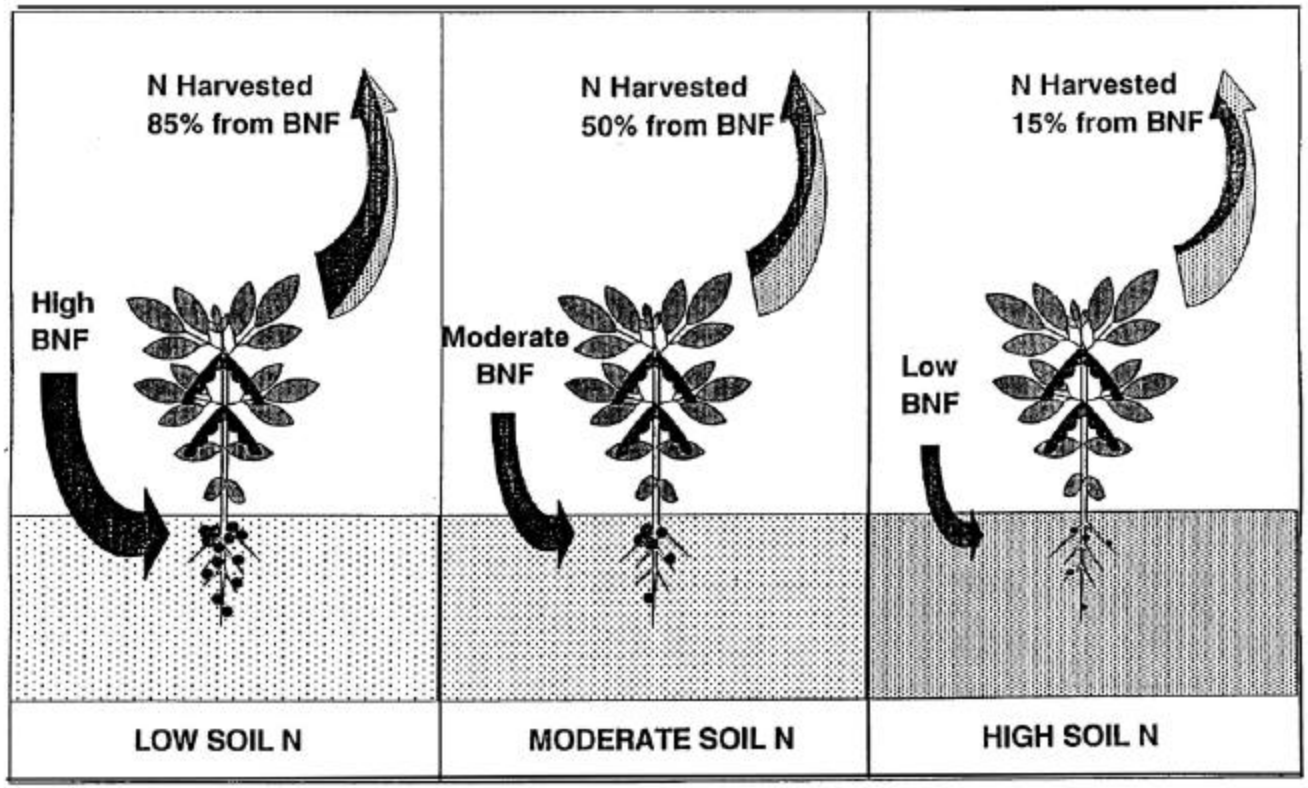
KEY CONCEPTS

The BNF symbiosis results from the complex processes of infection of roots by rhizobia, nodule development, nodule function and nodule senescence.

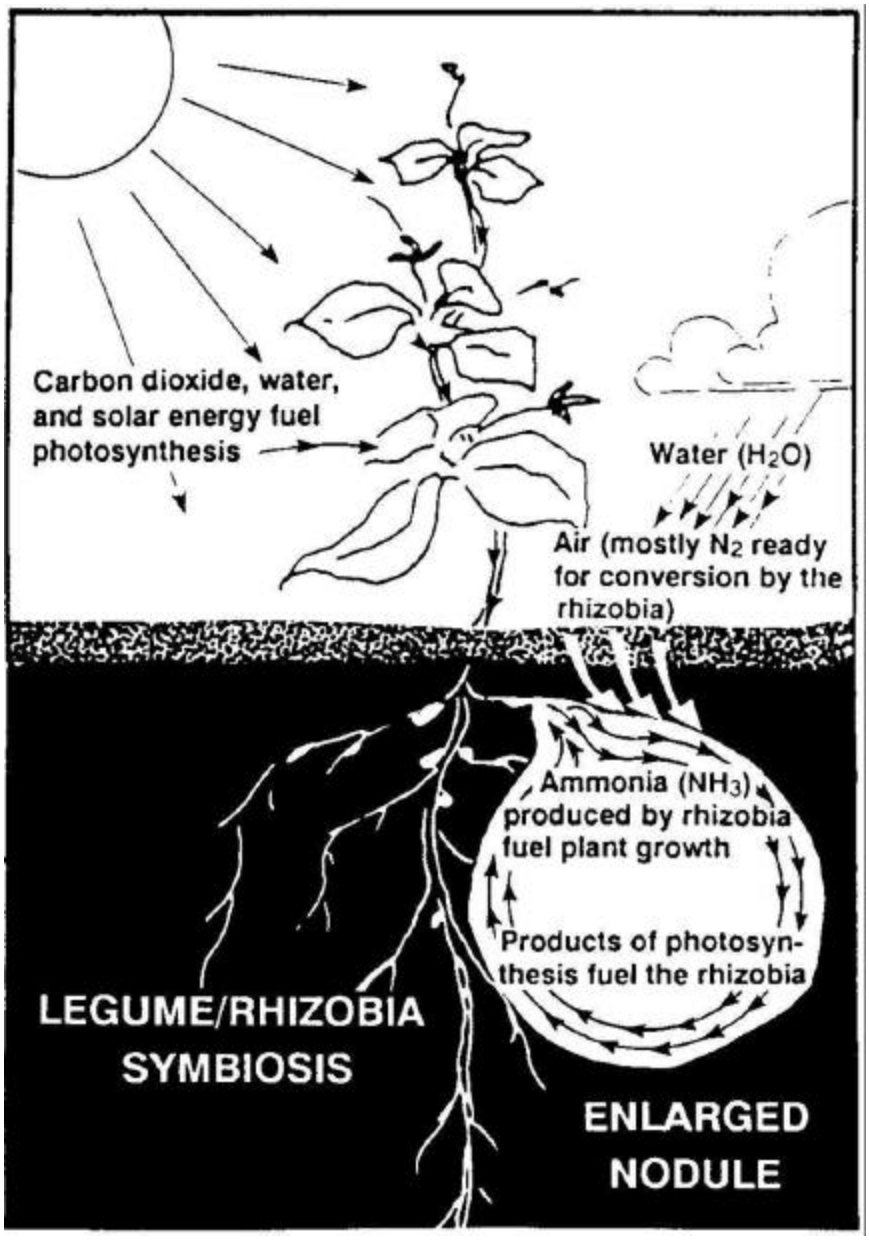
The amount of nitrogen that is fixed by a legume depends on several factors. The level of available soil nitrogen is probably the most important factor. The activity of BNF is at a maximum when soil nitrogen is minimal.

Legumes differ greatly in the amount of nitrogen they leave in the field for subsequent crops. The concepts of harvest index, nitrogen harvest index, and percent nitrogen from BNF are useful for estimating nitrogen Inputs from legumes and benefits of legume BNF to the crop system.

In addition to being grown directly for their seed, legumes are beneficial as rotational crops, green manure, cover crops, forage, and fuelwood.



INCREASING LEVELS OF SOIL NITROGEN REDUCE NODULATION AND THE AMOUNT OF NITROGEN OBTAINED FROM BIOLOGICAL NITROGEN FIXATION.


Carbon dioxide, water, and solar energy fuel photosynthesis
Water (H_2O)
Air (mostly N_2 ready for conversion by the rhizobia)
Ammonia (NH_3) produced by rhizobia fuel plant growth
Products of photosynthesis fuel the rhizobia
LEGUME/RHIZOBIA SYMBIOSIS
ENLARGED NODULE



Root nodules (top) and root gall (bottom) on *Medicago sativa*. Note that nodules are characterized by discrete attachments to the roots, organized growth and presence of lenticels.



Spherical root nodule of *Glycine max*.



Spherical nodules on *Desmodium sp*. Note the exterior pigmentation and the pronounced lenticel bands.





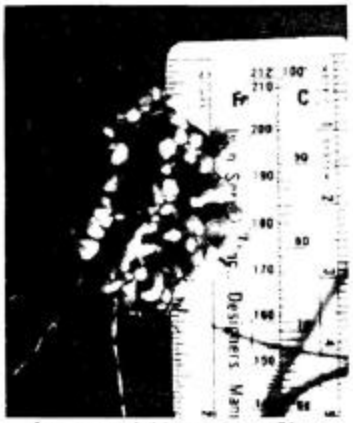
Elongate, branching nodules on *Trifolium repens*. Effective nodules (left) and ineffective nodules (right).



Cross section of elongate, branching nodules on *Onobryonis sativa* (safoin). Effective nodules (top) and ineffective nodules (bottom).



Young coraloid nodules on *Cicer arietinum* (Garbanzo).



Large coraloid nodule on *Cicer arietinum*.



Cross section of effective, young coraloid nodules of *Cicer arietinum*.

MODULE NUMBER 5

INOCULATION OF LEGUMES

SUMMARY

This module explains the proper handling and use of legume inoculant. It includes a brief introduction to inoculant production and the types of inoculants available, and provides suggestions on how to select good-quality inoculant. Inoculation application is explained in detail, including seed coating by various methods, soil inoculation, and the handling and storage of inoculant.

KEY CONCEPTS

- Many soil conditions make it necessary to inoculate legume crops to obtain maximum yields.
- The choice of methods for seed and soil inoculation depends on materials available and climate and soil conditions.
- The proper inoculant must be used with each legume.
- Inoculant contains living organisms that must be protected from heat and sun.
- Inoculant loses its effectiveness if not stored properly.
- Farmers do not get yield increases from inoculation if inoculant quality is poor.
- Inoculant production requires specialized equipment, knowledge, and skills.
- Different types of inoculants are produced for different needs.

WHAT IS INOCULATION?

Inoculation simply means bringing the appropriate rhizobia into contact with legume seeds or roots. In fact, farmers have been inoculating their legume seeds for centuries by collecting soil from fields that produce well-nodulated crops and transporting it to other fields where they wish to plant legumes. This method is difficult and time consuming because a great deal of soil needs to be transported, and it is also not very effective.

Modern inoculants contain live rhizobia that are grown in a laboratory and transported to the farmer as a liquid or solid substance. These inoculants have millions of rhizobia per gram, specifically selected to stimulate BNF in a particular legume crop. In 300 grams of a high-quality modern inoculant there are about the same number of rhizobia as in a 4-ton truckload of soil from a field with a successfully nodulated legume crop. See **Figure 5-1.** These modern inoculants are applied as seed coatings or incorporated into the soil like granular fertilizer. They may contain single or multiple strains of rhizobia. Inoculation is a fairly simple procedure. It is also generally much cheaper than fertilizer application and, unlike fertilizers, rhizobial inoculants have no negative side effects—it is impossible to

over-inoculate.

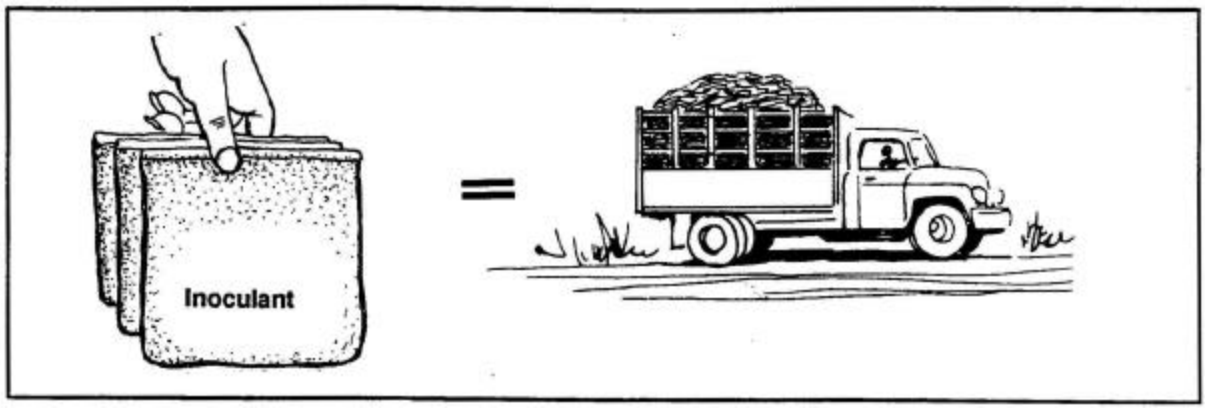


Figure 5-1. The truck contains 4 tons of soil from a field which had produced well nodulated legumes. the hand holds three 100 gram bags of rhizobia inoculant. There are the same number of living rhizobia in the three small bags as in the truck filled with soil.

There are several types of inoculants—liquid cultures, freeze-dried preparations, oil-dried preparations on talc or vermiculite, and liquid broth cultures mixed with a carrier material such as peat, charcoal, or lignite. The liquid cultures mixed with powered peat are the most popular type. Major producing countries are Australia, Brazil, New Zealand, Thailand, and the USA.

Inoculation is always recommended unless there is convincing evidence that inoculation is not necessary

Many soils contain rhizobia, but often these native rhizobia are not numerous enough or effective enough to stimulate BNF and increase yields. In addition, they are often not compatible with the specific legume crop the farmer wishes to plant. To obtain the full benefit of BNF in the species, it is necessary to **inoculate** legumes with compatible rhizobia.

Farmers should always inoculate when planting a legume species for the first time. Successful nodulation of a different legume, grown previously in the same field, does not ensure that the right rhizobia are present for the new crop. Even if the same legume was grown some years earlier, the rhizobial population in the soil may no longer be large enough for good nodulation. The best practice is simply to inoculate legume crops every season, particularly in climates with periods of drought or in acid or sandy soils—conditions that can kill rhizobia between crop cycles.

HOW ARE INOCULANTS PRODUCED?

Inoculants are produced by universities, government institutions, and private companies. The process requires specialized equipment plus considerable knowledge and skill in microbiology. Production starts with the selection of individual rhizobial strains that are effective with particular legumes. These strains are grown to high populations in the laboratory, then usually mixed into a carrier material such as ground peat, packaged, and distributed to farmers. We will briefly describe the steps in the production process.

Selecting and Growing Superior Rhizobia

Individual rhizobial strains are first isolated from legume root nodules and multiplied under artificial conditions (cultured) in the laboratory. Often inoculant producers test their strains for competitiveness and tolerance to stressful soil conditions such as low pH or high temperatures. Superior strains are identified in tests like the one shown in Module 3 (**Figure 3-4**), and further tested in the field.

The selected rhizobia are cultured in a liquid broth, called a **medium**, that supplies them with energy, a source of nitrogen, certain mineral salts, and growth factors. A sterile medium is commonly used, containing yeast extract, mannitol, and mineral salts (YM), with cane sugar or molasses sometimes substituted for the mannitol. It is essential that growth conditions be absolutely sterile.

Rhizobia grow best between 25° and 30°C. They require aeration, provided by shaking or bubbling sterile air. They are first cultured in a laboratory flask for three to seven days and then transferred to a larger fermentor. Fermentors vary in capacity from small glass vessels that can produce a few liters of rhizobia culture to steel fermentors with a capacity of several thousand liters. All have some provision for sterilization.

A fully grown culture should have a minimum of 1 billion (10^9) rhizobia per ml. Depending on the volume of inoculant broth and on the temperature, full growth may be reached in three days for bean rhizobia or five days for soybean rhizobia. At this point, the broth culture is ready to be mixed into a carrier.

Figure 5-2. Inoculant production using sterile carrier.

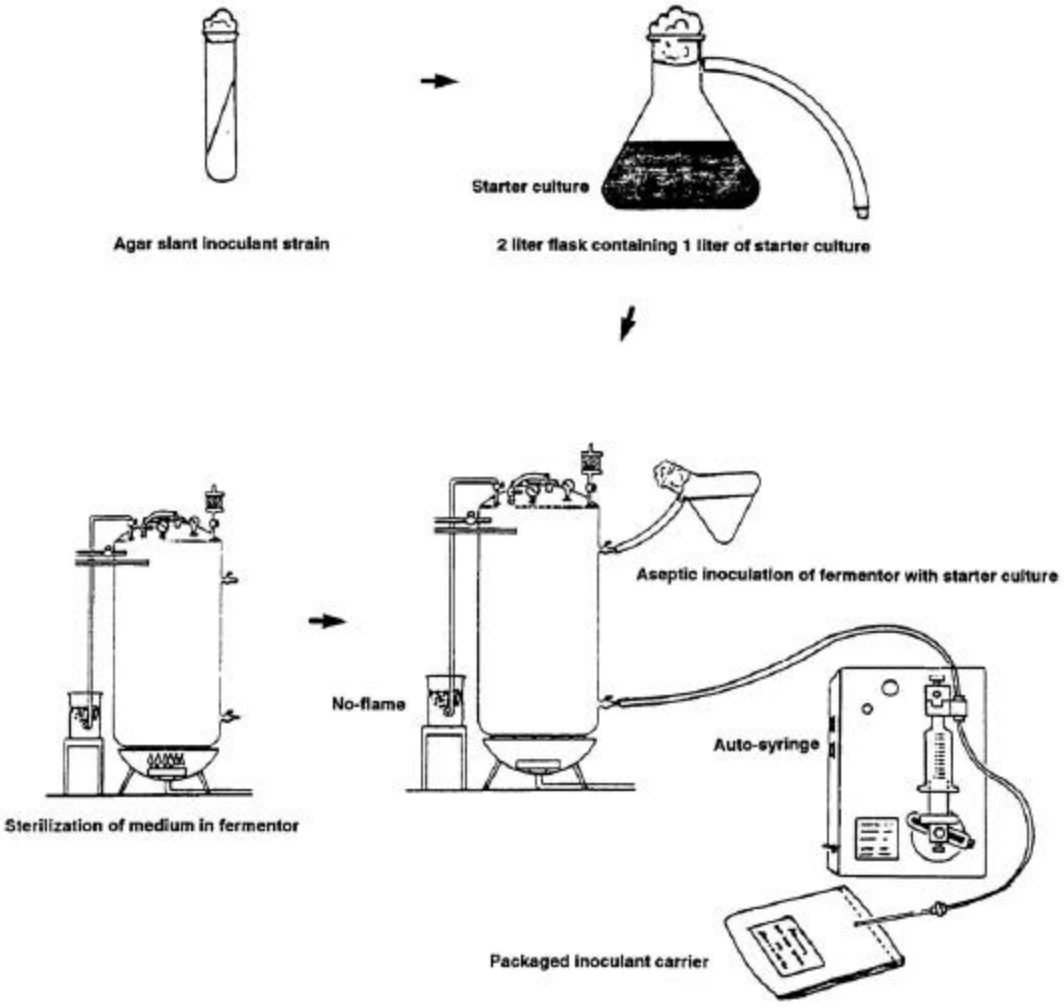
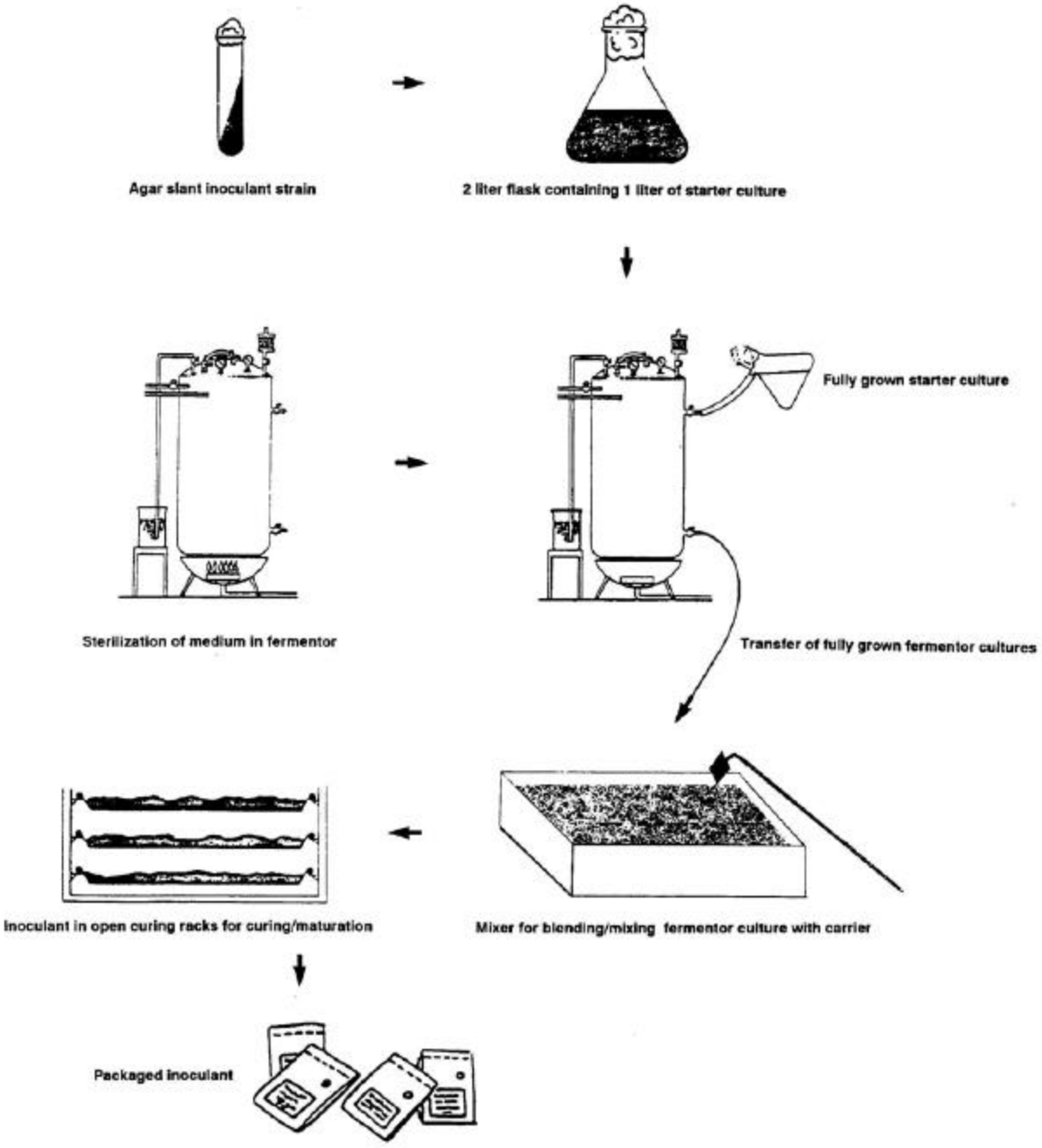


Figure 5-3. Inoculant production using non-sterile carrier.



Preparing Inoculant for Delivery to the Farmer

When a rhizobia culture has grown in the fermentor to maximum numbers, it is tested for purity and then mixed into a **carrier** and packaged for distribution to farmers. A good carrier can be mixed with one billion (10^9) live rhizobia per gram and will maintain 100 million (10^8) rhizobia per gram in good condition for at least six months. Ground peat is the most popular carrier, but other materials, such as lignite, filter mud, charcoal, soil, or bagasse, may be used when peat is not available. Each carrier material has special processing requirements, but all should have the following qualities:

1. Not toxic to rhizobia. A neutral pH is required.
2. High water-holding capacity.
3. Easily ground to a fine powder: Particle size is 10 to 40 microns for seed inoculants and 0.5 to 1.5 mm for granular inoculants.
4. Easily sterilized.
5. Inexpensive.

The rhizobia culture is mixed with the carrier at a rate of about 1 liter culture to 1 kg carrier. The final mixture will have a moisture content of 40 to 60% of the wet weight, depending on the carrier material. After mixing, the inoculant is allowed to cure for one to two weeks at 25° to 30°C to attain the maximum number of rhizobia. It is then packaged and labeled for distribution to the farmer. Some producers also offer inoculants in liquid carriers, but their availability is generally limited to North America and Europe.

Inoculant producers in Australia, New Zealand, Indonesia, Zambia, Kenya, and other countries produce inoculants with **sterile** carriers. With this process, the carrier, usually ground peat, is first packaged and then sterilized by autoclaving or gamma-irradiation. The rhizobial broth culture is injected into the packaged carrier with a syringe. Because sterilization eliminates any other microorganisms that might compete with the rhizobia, the number of rhizobia in a sterile carrier remains high for a long time. The expiration date is usually one year after production.

Most inoculant producers in the USA use **nonsterile** peat carriers. They mix the broth cultures of rhizobia with the carrier mechanically, cure the mixture in large open trays, and then package and seal it. This is a less expensive process, but inoculants produced in this way lose their effectiveness more quickly than inoculants in sterile carriers. Their expiration date is usually six months after production.

Quality Control

Inoculant quality control begins with the purity of the rhizobia in the fermentor. The broth culture must be protected from other microorganisms during production. Contaminants such as yeast or other bacteria inhibit growth of the rhizobia. Inoculant producers must monitor their cultures throughout the growth period, using several different techniques including those in Demonstrations 5-2 and 5-4. After the broth has been mixed with the carrier and allowed to mature, the inoculant is again tested to be sure that it contains

enough rhizobia. In some countries, government agencies also test inoculants to insure their quality for the farmers.

SELECTING GOOD INOCULANTS

Unfortunately, not all the inoculant produced is of good quality, and it is not always easy to distinguish good inoculants from poor ones. If possible, the extension agent should contact a government or university laboratory that is equipped to test the quality of the inoculant. Check the reputations of the various manufacturers and ask other users about their experience. Read the label carefully to select the right type of inoculant and to make sure the inoculant is fresh. Also be sure that the inoculant has been stored properly. Finally, you can conduct a **grow-out test** to compare the quality of various inoculants.

Qualities of a good inoculant

The ideal inoculant should have the following qualities:

1. **Effective on the legume**. It must have superior ability to form nitrogen-fixing nodules on the legume species and cultivars for which it is recommended.

2. **Competitive.** It must survive, infect the legume crop, and stimulate nodule formation even when other infective rhizobia are present in the soil.

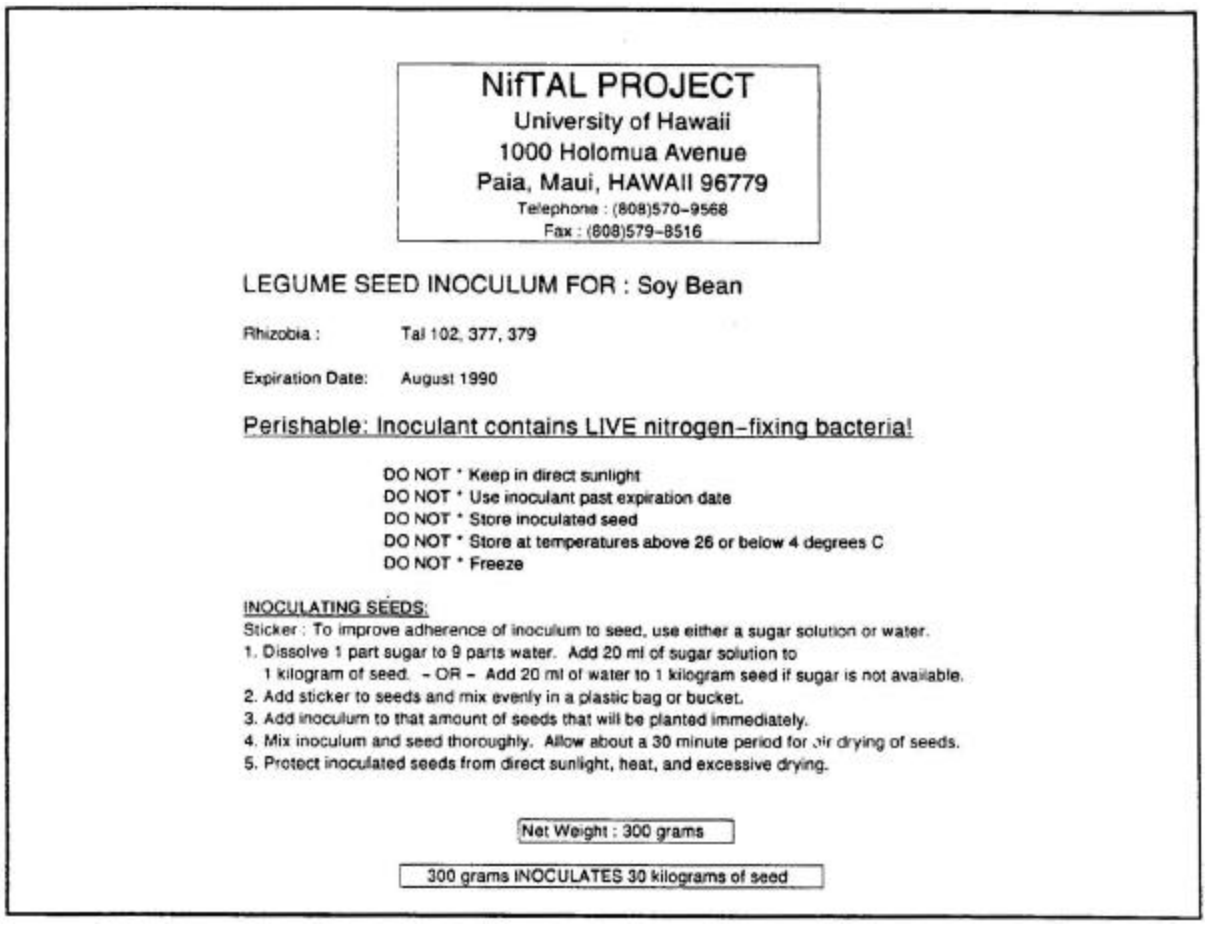
3. **Tolerant of stress**. It must stimulate nodulation and nitrogen fixation over a wide range of soil temperatures, acidity, alkalinity, and salinity conditions.

4. **Persistent in the soil**. Ideally, it should survive and grow in the soil between crops.

It is almost impossible to find an inoculant with all these qualities. However, any inoculant must contain superior rhizobia capable of producing nodules and fixing nitrogen on the plants for which it is designated.

Read the Label

The extension agent needs to read the label on the inoculant package carefully before recommending an inoculant to farmers. The label contains important information, such as the proper legume species for the inoculant and its expiration date. **Figure 5-4** gives an example of a label on an inoculant package.



NifTAL PROJECT
University of Hawaii
1000 Holomua Avenue
Paia, Maui, HAWAII 96779
Telephone : (808)570-9568
Fax : (808)579-8516

LEGUME SEED INOCULUM FOR : Soy Bean

Rhizobia : Tal 102, 377, 379

Expiration Date: August 1990

Perishable: Inoculant contains LIVE nitrogen-fixing bacteria!

DO NOT * Keep in direct sunlight
DO NOT * Use inoculant past expiration date
DO NOT * Store inoculated seed
DO NOT * Store at temperatures above 26 or below 4 degrees C
DO NOT * Freeze

INOCULATING SEEDS:
Sticker : To improve adherence of inoculum to seed, use either a sugar solution or water.
1. Dissolve 1 part sugar to 9 parts water. Add 20 ml of sugar solution to 1 kilogram of seed. - OR - Add 20 ml of water to 1 kilogram seed if sugar is not available.
2. Add sticker to seeds and mix evenly in a plastic bag or bucket.
3. Add inoculum to that amount of seeds that will be planted immediately.
4. Mix inoculum and seed thoroughly. Allow about a 30 minute period for air drying of seeds.
5. Protect inoculated seeds from direct sunlight, heat, and excessive drying.

Net Weight : 300 grams

300 grams INOCULATES 30 kilograms of seed

Figure 5-4. A sample inoculum label

The label should list the **legume species** for which the inoculant is effective. This information is crucial since, as we learned in Module 3, rhizobia will only stimulate nodulation and active BNF in their matched host legume species. It should also list the **name and address of the inoculant manufacturer**. Extension agents who become familiar with the use of inoculants can select products from manufacturers with good reputations and high standards of quality control.

The label should provide information on the **species of rhizobia** in the inoculant, and may also list the strain or strains used. Inoculants with **multiple strains** are usually effective over a wider range of legume species and soil conditions. Another crucial piece of information is the **expiration date**. The inoculant must be used before the expiration date.

The label must give **directions for use and rate of application.** BNF will only be successful if enough rhizobia are applied to each seed. The label should also give information on **how to store the inoculant**. Even if inoculant is used before the expiration date, it will not be effective if it has been improperly stored. Finally, the label should give the **net weight of the inoculant**. By comparing the recommended application rate and the net weight, the extension agent should be able to calculate the correct amount of inoculant

a farmer will need for the amount of seed to be planted.

Check Storage Conditions

Even a high-quality inoculant will lose its effectiveness if most of the rhizobia die due to improper storage. **Heat** is the most serious threat. Distributors should take care to refrigerate their inoculants or otherwise keep them cool. If an inoculant is exposed to heat during transport or if it is displayed in a hot store window, you can be sure that its quality has suffered.

Avoid Precoated Seed

In some countries, manufacturers distribute seed that has already been coated with inoculant. We do not recommend purchasing preinoculated seed. Their quality is often poor because rhizobia do not survive well on seeds. Farmers should inoculate their seed just before planting.

When in Doubt, Conduct a Grow-out Test

If you are in doubt about the quality of an inoculant or your need to choose from a number of inoculant producers, it is a good idea to conduct a **grow-out test**. Apply the inoculant at the recommended rate to a small number of legume seeds and grow these in pots or under other controlled conditions. Plant some seeds that are not inoculated. The seeds should be planted in soil that does not already contain the appropriate rhizobia. For example, take soil from a forest, a roadside, or a field under continuous cereal cropping, but not from a field where the same legume species has been grown. Observe whether the plants are growing well and have a healthy color. Dig the plants up when 50% are flowering—for soybean this will be about 30 days after planting. Compare the inoculated and uninoculated plants. Count the root nodules and cut a few open to check their color. Healthy nodules are pink to red inside. You can recommend the inoculant that produces healthy plants with large numbers of healthy root nodules.

Table 5-1 gives an example of a grow-out test performed in Ecuador. To make sure of the results, each brand of inoculant was tested twice, and results from the two tests were consistent. Nitragin and E.Z. performed well, Urbana performed moderately well, but Legume Aid, Noctin, and Dormal performed poorly. In the first test, the uninoculated plants had a few nodules. This indicates that there was some contamination, perhaps from rhizobia carried over from the other pots.

Table 5-1. Nodule number per soybean plants inoculated with various brands of inoculants.

	Ecuador, 1970*	
Inoculant Brand	**Boliche**	**Portoviejo**
	- - - - - - - - - - nodule no. per plant - - - - - - - - - - -	
Nitragin	31	23
E.Z.	23	26
Urbana	13	13
Legume Aid	1	5
Noctin	1	1
Dormal	1	1
No inoculation	3	0

Source: Data provided by INIAP.

SEED INOCULATION

Farmers should coat their seed with inoculant just before planting so that large numbers of rhizobia will be ready to start the infection process when the legume roots emerge. These rhizobia can then quickly infect the roots and start the process of nodulation.

Stickers

If a farmer simply dusts dry seeds with dry powdered inoculant, most of the inoculant will blow away before the seeds are planted. The inoculant needs a liquid or gummy **sticker** to bind it to the seed during the planting process. Popular sticker materials include gum arabic (mixed 40% in hot water), widely used in the Middle East and North Africa, carboxymethyl cellulose (4% in water), used most frequently in Australia, sugar (10% in water), corn syrup (10% in water), honey (10% in water), powdered milk (10% in water), evaporated milk (20% in water), mineral oil, or a vegetable oil such as peanut oil or soybean oil. Tests on soybean have shown that all of these can stick more than 100,000 live rhizobia to each legume seed, which is enough for good nodulation and nitrogen fixation.

Water is frequently used as a sticker, but it can be quickly absorbed by the seed and the inoculant can then blow away during planting. Materials that leave a sticky coating on the seed are better, such as oils or gum arabic. The sticker should not contain any substances that are harmful to the rhizobia or the seed. For example, one farmer used Coca-Cola as an inoculant sticker, but the high acidity killed the rhizobia.

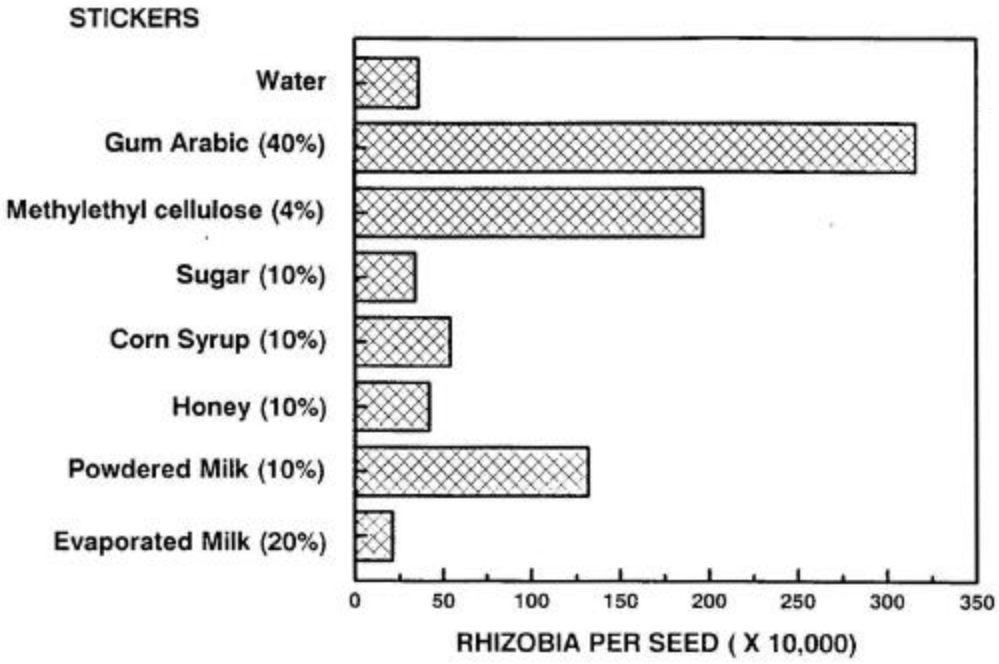


Figure 5-5. Effect of different stickers on the number of viable inoculant rhizobia on soybean seeds at time of planting. Adapted from Elegba and Rennie, Can. J. Soil Sci., 1984.

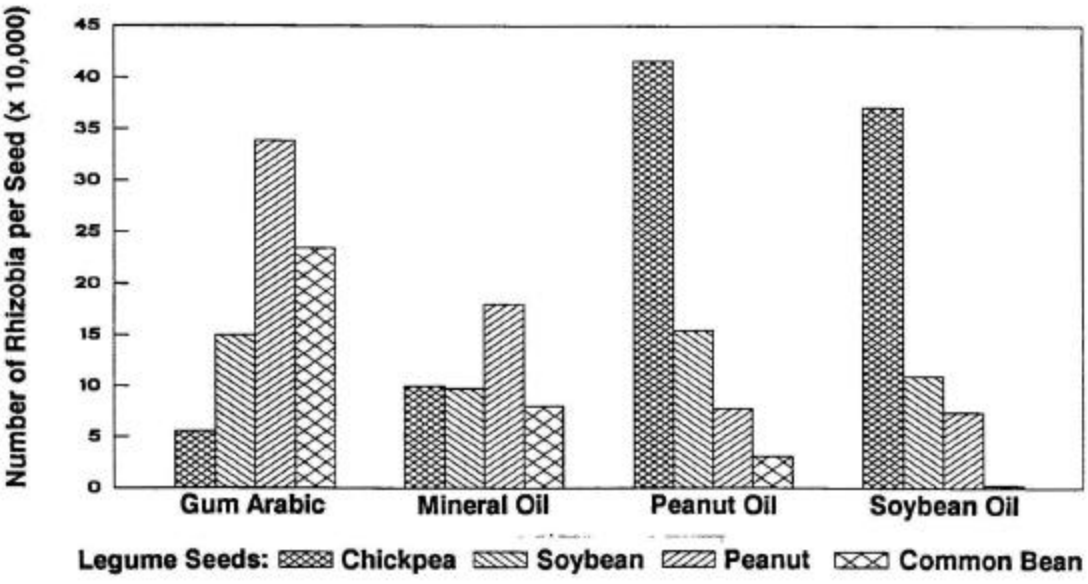


Figure 5-6. Survival of rhizobia on legume seeds after 3 days of storage at 34°C (Hoben, et al, 1991).

Table 5-2. Preparation of sticker material.

Slides	Concentration	Preparation
Gum Arabic	40% in water	Heat 100 ml water. Maintain just below boiling. Add 40 g of GA gradually while stirring. Allow to cool before use.
Carboxymethyl	4% in water	Add 4 g of carboxymethyl cellulose to 100 ml of cold water. Stir until dissolved.
Sugar	10% in water	Mix 10 g of sugar with 100 ml of cold water. Stir until dissolved.

The Dusting Method

Frequently farmers do not use any stickers. They simply mix dry powdered inoculants such as the peat-based type with dry seed without using any kind of liquid. This dry dusting is the poorest method of inoculation since dry inoculant does not adhere well to seed, and most of it will blow away during planting.

The Slurry Method

Seed can be inoculated by coating them with a slurry of inoculant and sticker solution. Just before planting, mix premeasured amounts of sticker solution and inoculant thoroughly to make a smooth liquid slurry. Add this to a weighed amount of seed and stir the mixture continuously until the seed is evenly coated. The mixing container should be twice as large as the volume of the seeds. A cement mixer is recommended for large amounts. **Figure 5-4** illustrates the slurry method of inoculation.

The rate of sticker solution mixed with the inoculant depends on the type of seed used. Smaller seeds require more sticker solution per seed weight than larger seeds because they have a larger surface area to be coated. The slurry should be added to the seeds in small amounts because too much sticker solution will cause the seeds to clump together or swell. **Table 5-2** gives the amount of sticker solution needed to inoculate 1 kg of seed of various legume species. For all species, 5 g of inoculant should be applied for each 1 kg of seed.

The Two-Step Method

In the **two-step method**, the sticker and inoculant are applied to the seed separately. In the first step, seeds are evenly coated with the sticker solution. In the second step, the powdered inoculant is added to the sticky seeds. About 10 times as many rhizobia can be bound to seed by the two-step method as by the slurry method.

With this method, it is particularly important to use the proper amount of sticker. Too much

sticker will make the seeds clump together and too little will cause uneven

coating with the inoculant. When coating a large number of seeds, the sticker should be added in small amounts until the seeds are evenly wet. The amount of inoculant to be added is not as critical. The general recommendation is to add 10 g of inoculant for each 1 kg of seed, but for soybean, for example, you may use 10 to 50 g of inoculant per kg of seed. **Table 5-3** provides guidelines.

Figure 5-7. Seed inoculation by the slurry method. a. Equipment necessary for the process: inoculant, water, seed, and a bowl for mixing water and inoculant; b. Adding water to inoculant; c. Mixing the "slurry" with seed; d. seed coated with dried inoculants; and e. A close-up view of coated and uncoated seed. Source: *Legume Inoculants and Their Use*. The two-step inoculation method.

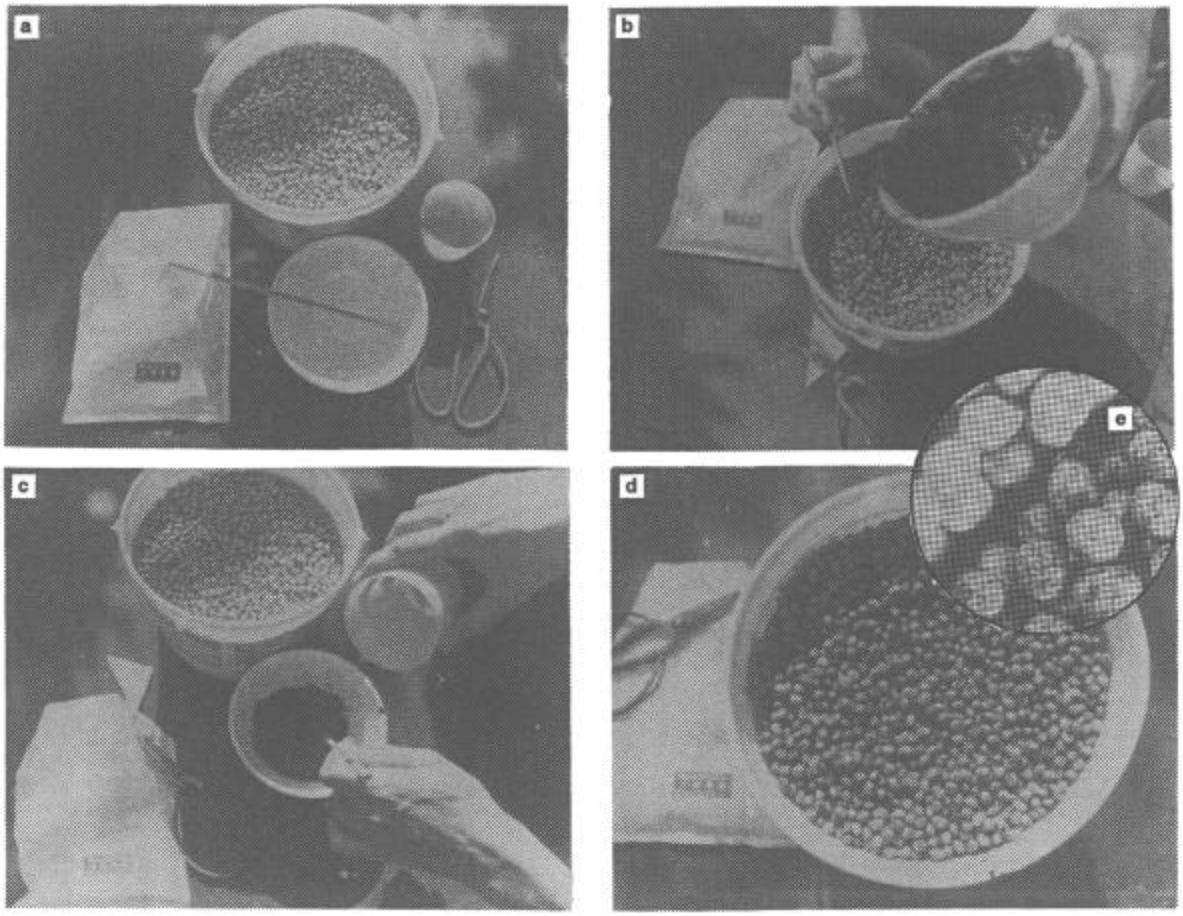


Table 5-3. Amounts of inoculant and sticker solution required for the slurry

inoculation of seeds of various size and resulting numbers of rhizobia per seed.

Legume species	Seeds (no/kg)[1]	Inoculant g/kg of seed	Sticker Solution ml/kg of seed	Rhizobia/ seed
Trifolium repens (white clover)	2000000	5	25	$2.5x10^3$
Medicago sativa (alfalfa)	500000	5	22	$1.0x10^4$
Desmodium intortum (Intortum)	440000	5	22	$1.1x10^4$
Stylosanthes hamada (stylo)	400000	5	22	$1.2x10^4$
Coronilla varia (crown vetch)	250000	5	22	$2.0x10^4$
Phaseolus atropurpureus (Siratro)	67000	5	20	$7.5x10^4$
Vigna radiata (green gram)	25000	5	20	$2.0x10^5$
Cajanus cajan (pigeon pea)	17000	5	20	$3.0x10^5$
Vigna unguiculata (cowpea)	10000	5	15	5.0x10
Glycine max (soybean)	5000	5	15	$1.0x10^6$
Phaseolus vulgaris (common bean)	2400	5	10	$2.1x10^6$
Cicer arietinum (chickpea)	2000	5	10	$2.5x10^6$
Arachis hypogaea (peanut)	2000	5	10	$2.5x10^6$
Vicia faba (broad bean)	1250	5	7	$4.2x10^6$

Note: Legumes are arranged in ascending order of size
[1]Approximate values
Inoculant of $1x10^9$ cells/gram
Adapted from *Legume Inoculants and Their Use*, FAO/NifTAL

Figure 5-8 illustrates an easy two-step coating procedure using a plastic bag. First, prepare the sticker solution. Gum arabic should be prepared one day ahead of time; other stickers may be prepared immediately before application. Place 1 kg of seed in a plastic bag. Add the sticker and inflate the bag. Twist the bag shut to trap as much air as possible and swirl the bag for one minute or more until all the seeds are evenly wet. Open the bag, add the inoculant, inflate the bag again, and shake gently. Stop as soon as all the seeds are coated evenly, as too much shaking will break down the coating. Pour the seed on a clean surface in the shade and allow to air dry before planting. This plastic-bag method with batches of seed more than 3 kg, the inflated bag should be rolled on the ground rather than shaken.

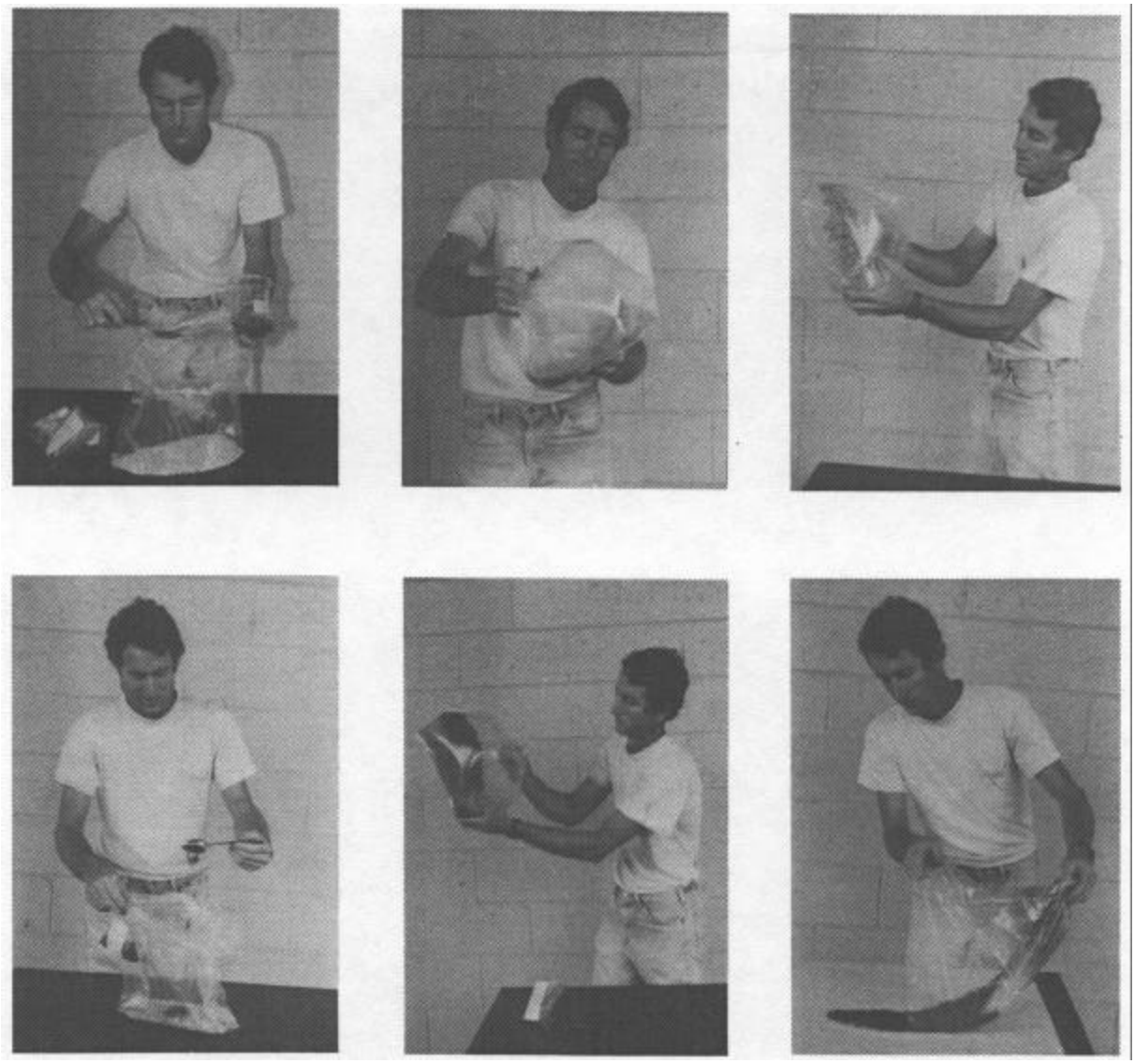


Figure 5-8. Seed coating by the two-step method.

Table 5-4. Amount of sticker recommended for the inoculation of seed of various sizes by the two-step method.

		Stickers (ml)/kg seed			
Legume species	**Seeds (no/kg)[1]**	**Sugar 20% solution**	**M-E Cellulose**	**Gum Arabic**	**Rhizobia/ seed**
Trifolium repens (white clover)	2000000	50	38	25	$5.0x10^3$
Medicago sativa (alfalfa)	500000	44	33	22	$20x10^4$
Desmodium intortum (Intortum)	440000	44	33	22	$2.2x10^4$
Stylosanthes hamada (stylo)	400000	44	33	22	$2.4x10^4$
Coronilla varia (crown vetch)	250000	44	33	22	$4.0x10^4$
Phaseolus atropurpureus (Siratro)	67000	42	32	21	$1.5x10^5$
Vigna radiata (green gram)	25000	40	30	20	$4.0x10^5$
Cajanus cajan (pigeon pea)	17000	40	30	20	$5.9x10^5$
Vigna unguiculata (cowpea)	10000	30	23	15	$1.0x10^6$
Glycine max (soybean)	5000	20	15	10	$2.0x10^6$
Phaseolus vulgaris (common bean)	2400	20	15	10	$4.2x10^6$
Cicer arietinum (chickpea)	2000	20	15	10	$5.0x10^6$
Arachis hypogaea (peanut)	2000	20	15	10	$5.0x10^6$
Vicia faba (broad bean)	1250	14	11	7	$8.3x10^6$

[1]Approximate values
Inoculant is used at the rate of 10g/kg of seed
Adapted from *Legume Inoculants and Their Use.* FAO/NifTAL

Larger quantities of seed may be inoculated in a tumbler-type mixer such as a cement mixer. When using a cement mixer, check that the seeds are evenly coated with sticker before adding the inoculant. If the seeds clump together or stick to the wall of the mixer, stop the machine, break up the clumps, and scrape the mixer walls. Immediately after coating, spread the seeds out on a clean surface and allow them to dry in the shade before sowing.

Seed Pelleting

It is sometimes advantageous to coat inoculated seed with a protective layer of powdered lime or phosphate, for instance:

- **When adverse weather conditions cause delayed sowing**: Pelleting can prolong the survival of rhizobia on the seed until sowing.
- **When the soil is hot and dry**: When seeds must be sown in dry or hot soils, the protective pellet may help preserve both the rhizobia and the seed until conditions are suitable for germination. This protection is especially important when seeds are broadcast.
- **When insects are a problem**: In some areas, pelleting is used to protect seeds from insects, especially seed-gathering ants.
- **When soils are very acid**: Lime pelleting can help protect rhizobia from highly acid soils or acid fertilizers.

First, the inoculant is applied as a slurry or by the two-step method using a sticker solution as an adhesive. The lime or phosphate should be ground to a very fine powder and sifted through a screen to remove lumps. Add the powder immediately after inoculation while the seeds are still wet and mix it quickly until the seeds are thoroughly coated. The pelleted seeds will appear dry, but they should be spread out on a clean surface in a cool, shaded place to allow the pellet to solidify before sowing. **Table 5-4** gives recommended lime applications for seed of different species.

Table 5-5. Amount of powdered limestone required to pellet legume seeds after inoculation by the slurry method.

Legume Species	Seeds (number/kg[a])	Sticker (ml/kg seed)	Lime or Phosphate (g/kg seed)	Rhizobia (number/seed[b])
Trifolium repens (white clover)	2,000,000	42	400	5.0×10^3
Medicago sativa (alfalfa)	500,000	42	400	2.0×10^4
Desmodium intortum (intortum)	440,000	42	400	2.2×10^4
Stylosanthes hamada (stylo)	400,000	42	400	2.4×10^4
Coronilla varia (crown vetch)	250,000	42	400	4.0×10^4
Phaseolus atropurpureus (siratro)	67,000	40	350	1.5×10^5
Vigna radiata (green gram)	25,000	38	350	4.0×10^5
Cajanus cajan (pigeon pea)	17,000	38	250	5.9×10^5
Vigna unguiculata (cowpea)	10,000	17	200	1.0×10^6
Glycine max (soybean)	5,000	17	200	2.0×10^6
Phaseolus vulgaris (common bean)	2,400	16	200	4.2×10^6
Cicer arietinum (chickpea)	2,000	16	200	5.0×10^6
Arachis hypogaea (peanut)	2,000	16	200	5.0×10^6
Vicia faba (broad bean)	1,250	15	200	8.3×10^6

[a]Approximate values
[b]Assuming that inoculant contains 10^9 rhizobia/g and is applied at a rate of 10g/kg of seed.
Source: Adapted from FAO/NifTAL, 1984. *Legume Inoculants and Their Use.* Rome: FAO,72 pp.

SOIL INOCULATION

Seed coating is not always the best way to inoculate. Some inoculants are designed to be placed directly into the soil. This practice is recommended under the following conditions:

1. **When seeds are precoated with pesticides or herbicides.** These chemicals are toxic to rhizobia. Of the fungicides listed in **Table 5-5**, Thiram is the least toxic, but even this chemical can be harmful under some conditions. Soil inoculation is recommended for seed treated with these fungicides. Insecticides for legume crops are usually distributed in the furrows as granules. When applied in this way, they are not usually harmful to rhizobia.

2. **When planting in hot, dry soil.** If legume seeds are planted in hot, dry soil and must wait for rain before they germinate, the rhizobia used to coat them are likely to die. Under these conditions, the rhizobia will survive better if the inoculant is placed in the soil below the seeds.

3. **When seed inoculation has failed.** Soil inoculation can be used to save a crop that for some reason has failed to nodulate. The inoculant may be sprayed on the soil surface just before irrigation or rain.

4. **When very large numbers of rhizobia are needed.** Soil inoculation can add more rhizobia to a field than possible with seed inoculation. Soil inoculation may be necessary, for instance, when the introduced rhizobia must compete with a large population of native rhizobia that form nodules but are not effective nitrogen fixers. Soil inoculation may also be necessary if the quality of the inoculant is known to be poor and the farmer needs to increase the application rate.



Figure 5-9. A side view of soil inoculation. Notice that the inoculant granules are placed below the seed.

Table 5-6. Fungicides known to be toxic to rhizobia.

Captan	N-trichloromethylthio-4 cyclohexane-1, 2 dicarboximide
Carboxin	5,6 dihydro-2-methyl-N-phenyl-1,4 oxathiin-3 carboxamide
Chloramil	2,3,5,6 tetrachloro-1,4 benzoquinone
PCNB	Pentachloromitrobenzene
Thiabendazole	"Tecto" 2-(4 thiazolyl)-benzimidazole
Thiram	Tetramethyl-thiuram-disulphide

Table 5-7. Most commonly used insecticides

Carbofuran	2,3-dihydro-2, 2-dimethyl-benzofuranyl methyl-carbamate
Phorate	0-0 diethyl S-(ethyltrio)-methyl phosphorodithiate
Aldcarb	2-methyl-(emthylthio) propionaldehyde-0-(methyl-carbomoyl) oxime

Preparing Soil Inoculant

Commercial producers sometimes market inoculants with a granular peat carrier specifically designed for soil inoculation. The particle size is 0.5 to 1.5 mm. If commercial granular inoculants are not available, seed inoculant can easily be converted into soil inoculant. A dry type may be made by mixing 100 g of peat inoculant with 10 kg of fine sand. Place the dry, cool sand in a bucket, add the inoculant while stirring, and keep stirring until the mixture is a uniform color. Mixing the seed inoculant with sand makes it easier to handle and apply in the field: 1 kg of sand inoculant is enough to inoculate about 100 m of row.

If sand is not available, the seed inoculant can be mixed with sifted soil. A liquid soil inoculant can also be prepared by making a suspension of a 100 g seed inoculant in 10 liters of water.

Applying Soil Inoculant

Dry soil inoculant should be placed in the furrow before planting or with the seed. It should be place below the seed as shown in **Figure 5-6**. Commercial granular inoculant can be applied with a simple mechanical seed applicator.

Dry inoculants can also be broadcast in fields where pasture legume seeds, such as clover, alfalfa, siratro, and stylosanthes, have been sown by broadcasting. The inoculant should only be broadcast when the soil is moist or immediately before irrigation.

Liquid inoculant needs to be stirred continuously to keep the rhizobia evenly distributed. Application is easy with hand-held equipment as shown in **Figure 5-7**. This simple applicator consists of a canister with an outflow at the bottom to which a flexible tube is attached. A straight stick is taped to the tube to help the operator direct the flow of inoculant into the furrow. Alternatively, a pesticide applicator may be used, but must be washed carefully to remove all traces of poison. A watering can may be used, but the sprinkler should be replaced with a smaller outflow tube to direct the flow of inoculant.



Figure 5-10. Inoculant applicator. Liquid inoculant (inoculant mixed with water) can be applied before planting using this method.

A liquid inoculant applicator can also be mounted on the shaft of an oxplow directly behind the plowshares. A rock suspended inside the inoculant container will keep the liquid well stirred as the plow moves along the furrows. Seeds may also be dispensed from a container attached to the plow shaft.

Inoculating Tree Legumes

If tree seedlings are grown in plastic growth tubes (dibble tubes) or other containers in a nursery, dry inoculant can be mixed with the planting medium before the seeds are sown. Mix at a rate of 10^7 rhizobia per seedling or 1 g of inoculant per 100 seedlings. Seed inoculant mixed with water can also be poured into the seedling containers. Mix 1 g of dry inoculant with 1 liter of water and add 10 ml to each tube. See NifTAL's *Inoculating tree seeds and seedlings with rhizobia* (1990) for more information about inoculating trees.

If the seedlings are not well nodulated by the time of transplanting, 10 ml of diluted seed inoculant can again be watered into the tubes. Inoculant can also be mixed with soil in the planting hole or watered in at transplanting.

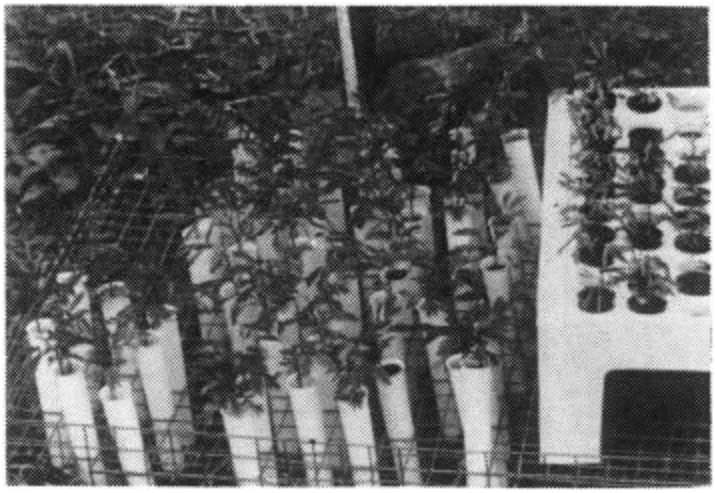


Figure 5-11. These trees were planted in dibble tubes for transplanting.

SUMMARY OF INOCULATION METHODS

Seed Inoculation by Slurry Method

1. Prepare sticker.
2. Make sufficient amount of slurry (**Table 5-2**).
3. Place seeds in a suitable container and add slurry (**Figure 5-4**).
4. Mix with a stick or by hand until the seeds are evenly coated.
5. Spread seeds on a clean shaded surface and allow them to dry.

Seed Inoculation by the Two-Step Method

1. Prepare sticker.
2. Weigh seeds and place them in a plastic bag or bucket (**Figure 5-5**).
3. Add sticker (**Table 5-3**).
4. Inflate bag to at least four times the volume of the seeds and shake vigorously.
5. Open bag and add preweighed inoculant.
6. Close bag, inflate, and shake gently for one minute or until seeds are well coated.
7. Spread seeds on a clean shaded surface and allow them to dry.

Seed Pelleting by Slurry Method

1. Inoculate seeds according to slurry method, but increase the amount of sticker solution and inoculant as listed in **Table 5-4**.
2. Add lime or phosphate in amounts listed in **Table 5-4** immediately after inoculation while seeds are still wet.
3. Mix until the seeds are evenly coated.
4. Spread seeds on a clean shaded surface and allow them to dry.

Seed Pelleting by Two-Step Method

1. Inoculate seeds according to two-step method.
2. Add lime or phosphate in amounts listed in **Table 5-4** immediately after inoculation while seeds are still wet.
3. Inflate bag and shake gently until seeds are evenly coated.
4. Spread seeds on a clean shaded surface and allow them to dry.

Soil Inoculation by Dry Application

1. Add inoculant to sand or sifted soil at a ratio of 10 g of inoculant to 1 kg of sand or soil.
2. Mix thoroughly by shaking or with a stick.
3. Apply to the furrow at the rate of 1 kg per 100 m row.

Soil Inoculation by Wet Application

1. Add 10 g of inoculant to 1 liter of water in an applicator canister (**Figure 5-7**) or watering can.
2. Mix thoroughly by stirring.
3. Apply to the furrow at the rate of 1 liter per 100 m row.

INOCULANT STORAGE AND HANDLING

Storing Inoculants

Always remember that **inoculants contain living organisms**. They should be treated with care. Most important, they must be kept away from sun and heat. When ordering inoculants, it may be helpful to inform the producer of the climatic conditions in your area so that adequate protection during shipping can be arranged. Exposing an inoculant to sun or heat for even a few hours—for instance on the shipping dock, in a hot shed, or in a hot car—will severely reduce the number of live rhizobia and thus reduce the effectiveness of the inoculant.

Generally, rhizobia survive in cool temperatures. Farmers, extension agents, and local distributors should store inoculants in a refrigerator if at all possible, but never in a freezer since freezing will kill the rhizobia. The ideal temperature is between 4◦ and 26◦C. If refrigeration is not available, the inoculant can be kept for a short time in a cool, shady spot indoors; an underground cellar or cool cave is suitable for longer-term storage. Inoculant can also be stored for up to six months in a well-sealed ceramic jar and buried in a shady spot underground. The jar should be covered with a thick wooden lid to serve as protection as well as insulation from heat.

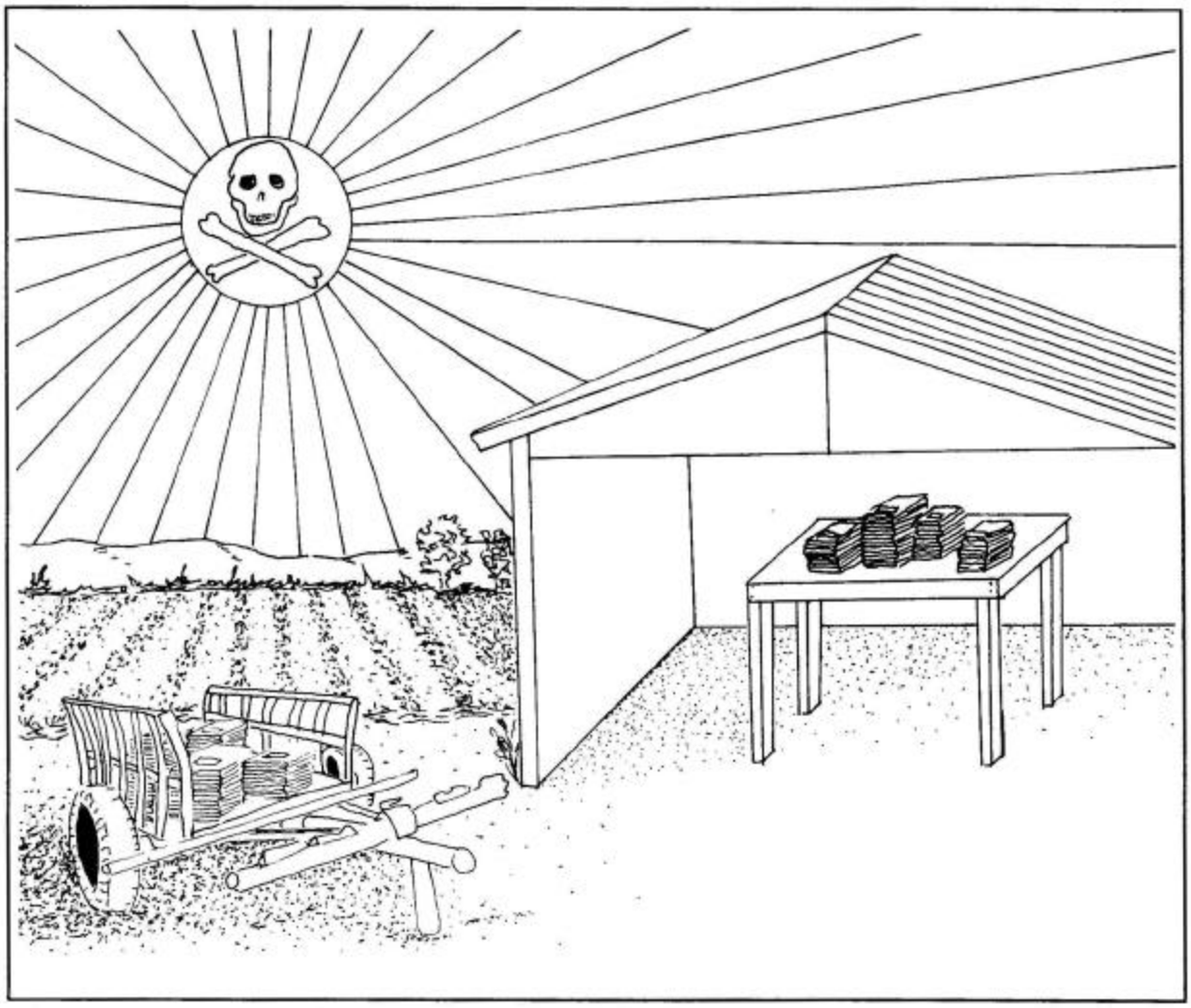


Figure 5-12. It is too hot to store inoculant in direct sunlight or in a metal roofed building.

In a trial at NifTAL, two inoculants—one for soybean and one for chickpea—were stored at different temperatures and checked for their effectiveness. Both survived well when stored at 28◦C, but the rhizobia died after one week of storage at 42◦C. After eight weeks at 37◦C, only 1 in 100 soybean rhizobia were still alive; among the chickpea rhizobia, only 1 in 10,000 survived.

Handling Inoculants in the Field

For best results, inoculant or inoculated seeds should be carried to the field in insulated coolers containing ice. If coolers are not available, the inoculants should be wrapped in moist towels and carried in a basket covered with a wet towel. In the field, the inoculant container should be kept in the shade of a tree or umbrella.

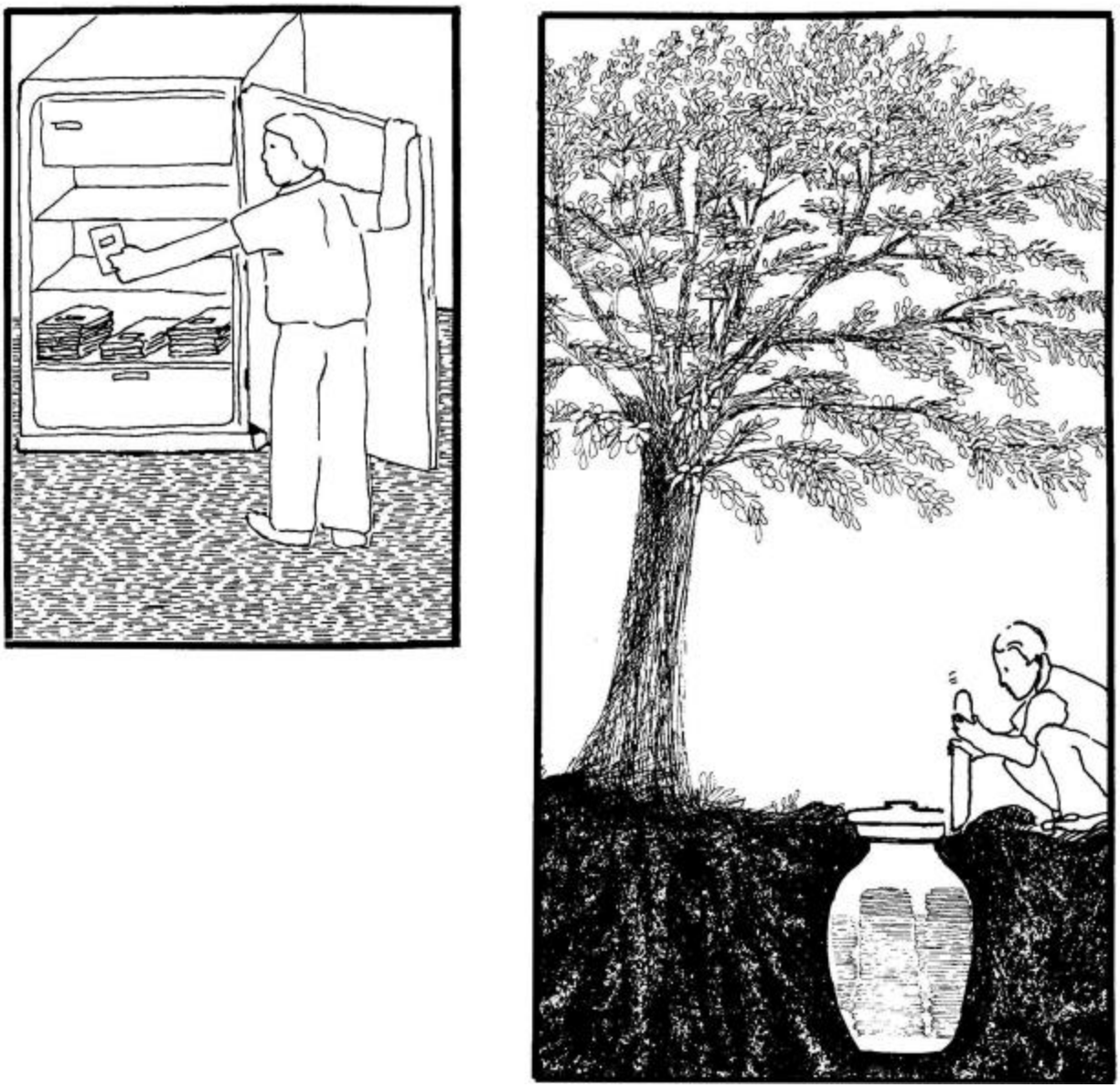


Figure 5-13. It is best to store inoculants in cool places. Refrigeration between 4° and 26° C is ideal. However, using a buried urn in a shady place will allow good storage for at least 6 months.

Seeds should be inoculated in a cool shady place on the day of planting. The inoculated seeds should be planted in moist soil and covered immediately so the rhizobia are not exposed to the sun. If possible, the field should be irrigated immediately after planting. Nodulation will be best if the seeds germinate right away and the roots come quickly into contact the inoculant.

If there is any unused inoculant, the package can be sealed with tape and stored in a cool place. If for some reason it is not possible to plant the seeds immediately after inoculation, they should be stored in a cool place and planted as soon as possible. Storing precoated seeds for more than a day or two is not recommended because the rhizobia do not survive long on the seed.

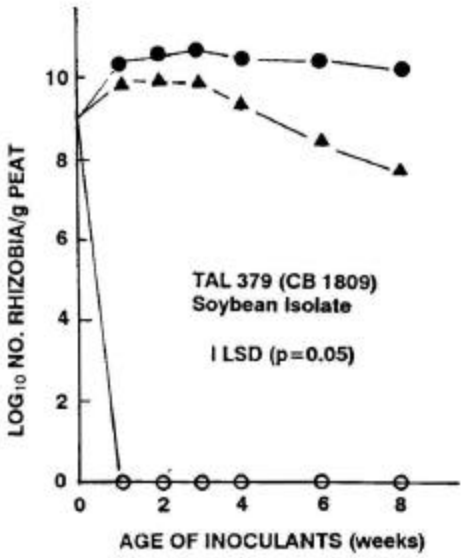


Figure 5-14. Survival of soybean rhizobia at three temperatures.

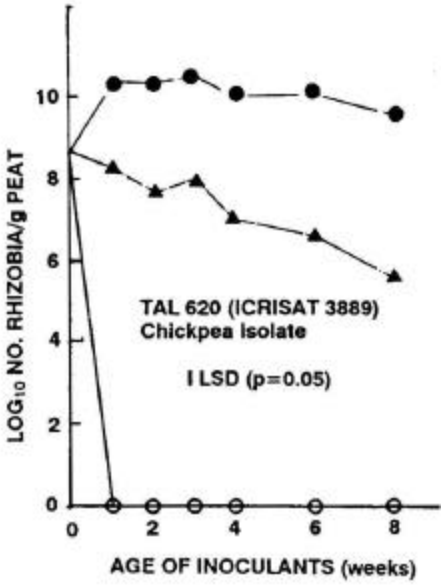


Figure 5-15. Survival of chickpea rhizobia at three temperatures.

Inoculation Rates and Costs

The total cost of inoculating a legume crop with rhizobia depends primarily on the cost of the inoculant and the cost of the extra labor required. These factors vary from country to country and from region to region, but in most cases the cost of inoculation is a very small proportion of total production costs, and the benefits are usually high. **Table 5-5** shows the amount of inoculant recommended to inoculate enough legume seed to plant one hectare, using the slurry method. The precise amount of inoculant required will vary with planting density, seed size, the quality of the inoculant, and soil and climatic conditions. The figures in **Table 5-6** are minimum recommended rates based on the assumption that there are at least 10^9 rhizobia per gram of inoculant. The two-step method uses at least twice as much inoculant, while soil inoculation requires a minimum of 1.6 kg of inoculant per hectare.



Figure 5-16. Inoculants should be transported to the field in a cool container. Three farmers are carrying inoculant in a basket covered with a wet cloth.

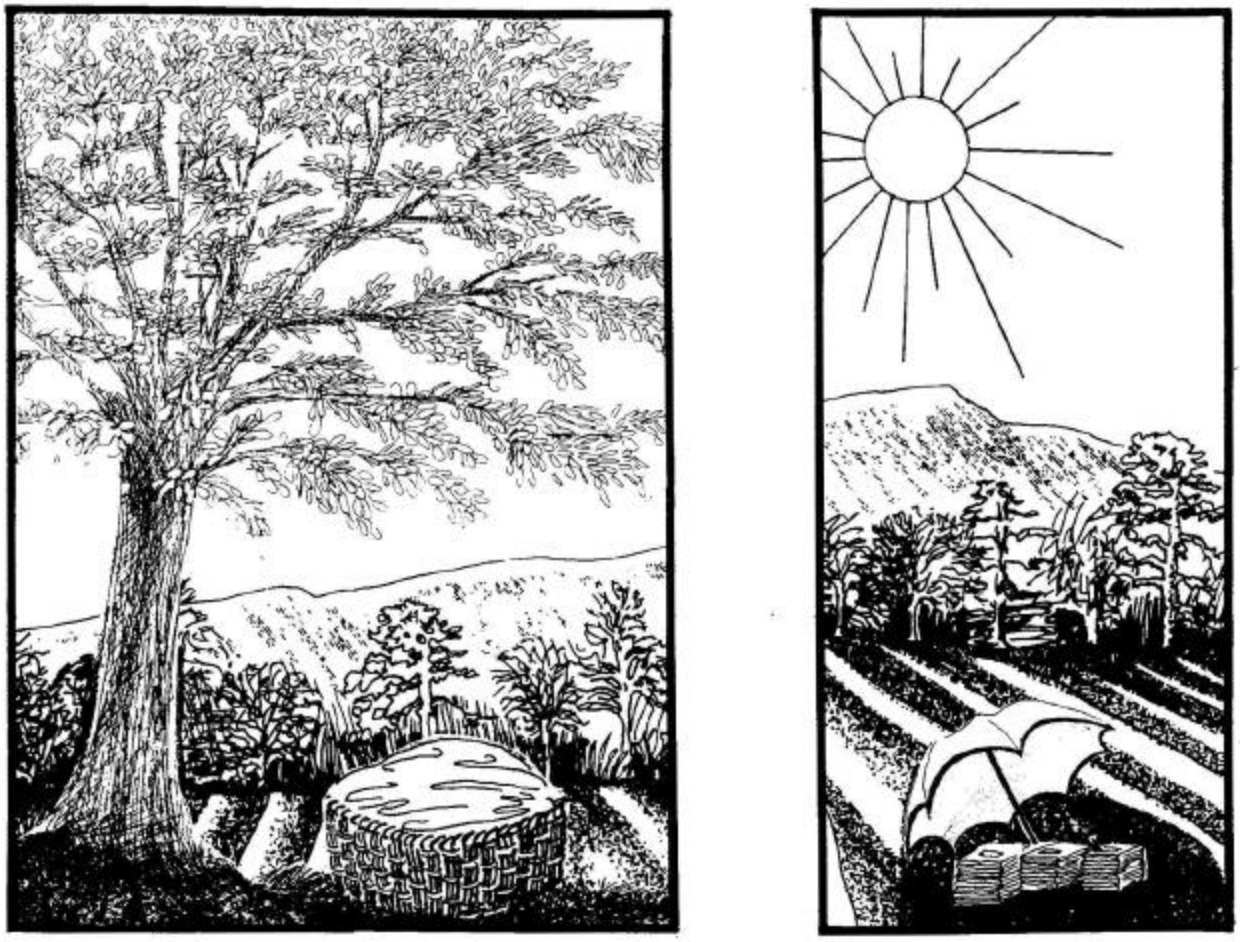


Figure 5-17. This inoculant is protected from the sun by an umbrella. Inoculant may also be placed under a shady tree.

Farmers want to know what benefits they can expect from using rhizobial inoculant. One simple way to evaluate the benefits of BNF is to estimate the amount of nitrogen fixed by an inoculated legume crop and to calculate the cost of applying that much nitrogen as fertilizer. Because crops do not use nitrogen fertilizer as efficiently as they use nitrogen produced by BNF, it would take at least 200 kg of nitrogen fertilizer to produce the same yield as that produced by 100 kg of nitrogen fixed through by BNF. If fertilizer costs US$100 for 200 kg of nitrogen ($0.50/kg) and the inoculant to fix 100 kg of nitrogen costs $4.00, then the cost/benefit ratio of inoculation would be 25.

$$\frac{200 \text{ X } 0.5}{4} = 25$$

This calculation assumes that a similar amount of labor is required to inoculate a crop or to apply fertilizer. Assuming that no native rhizobia present are in the soil, a cost/benefit ratio of 25 is a good estimate for soybean inoculation. For smaller-seeded legumes that require less inoculant per plant, the cost/benefit ratio can be much higher.

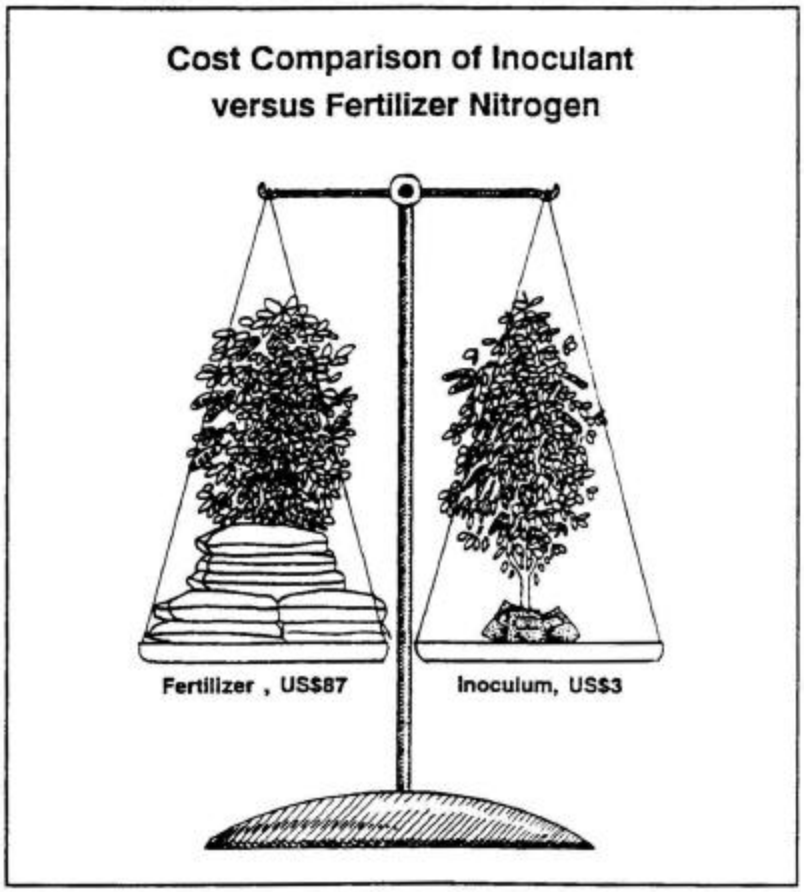


Figure 5-18. This scale shows the dramatic difference between the cost of chemical fertilizers and rhizobial inoculants.

Table 5-8. Approximate amount of seed and inoculant required for various legumes.

Legume species	Seeds (no/kg)[1]	Rhizobia/Seed	Kg Seeds/ha	Inoculant g/ha
Trifolium repens (white clover)	2000000	$2.5x10^3$	4	20
Medicago sativa (alfalfa)	500000	$1.0x10^4$	16	80
Desmodium intortum (Intortum)	440000	$1.1x10^4$	11	55
Stylosanthes hamada (stylo)	400000	$1.2x10^4$	16	80
Coronilla varia (crown vetch)	250000	$2.0x10^4$	20	100
Phaseolus atropurpureus (Siratro)	67000	$7.5x10^4$	38	190
Vigna radiata (green gram)	25000	$2.0x10^5$	76	380
Cajanus cajan (pigeon pea)	17000	$3.0x10^5$	11	55
Vigna unguiculata (cowpea)	10000	$5.0x10^5$	65	325
Glycine max (soybean)	5000	$1.0x10^6$	98	490
Phaseolus vulgaris (common bean)	2400	$2.1x10^6$	35	175
Cicer arietinum (chickpea)	2000	$2.5x10^6$	33	165
Arachis hypogaea (peanut)	2000	$2.5x10^6$	44	220
Vicia faba (broad bean)	1250	$4.2x10^6$	87	435

[1]Approximate values
Inoculant of $1x10^9$ cells/gram
Adapted from *Legume Inoculants and Their Use.* FAO/NifTAL.

REMINDERS

Do:

- Use the correct inoculant for each legume. Check the label for the species you are planting.
- Protect inoculant from sun and heat to keep it alive. The ideal storage temperature is between 4◦ and 26◦C.
- Store inoculant in closed bags.
- Use a sticker when inoculating seeds.
- Use the recommended amount of inoculant. Always use at least 5 g per kg of seeds.
- Inoculate seeds just before planting.
- Apply dry soil inoculant when the soil is moist or just before irrigation.
- Cover furrows immediately after planting inoculated seeds.

Don't:

- Expose inoculants to temperatures above 30◦C.
- Use inoculants after their expiration date or after they have been exposed to high temperatures.
- Let inoculants dry out.
- Apply inoculants to seeds without sticker.
- Mix fertilizer or pesticides with inoculated seeds.
- Apply inoculants to the surface of dry soil.
- Plant commercially preinoculated seeds.

REVIEW AND DISCUSSION

1. A group of farmers have stored their inoculant in an equipment shed for two weeks. You enter the shed and find that the temperature is above 40◦C. What do you recommend?
2. A farmer mentions that to save labor she is planning to apply dry inoculant to her seed without sticker. Explain the alternatives and discuss the advantages and disadvantages in relation to her concerns about the need to save labor.
3. Another farmer tells you that he does not think inoculation is necessary. After growing cowpeas for several years, he inoculated his crop last year and the yield was no different than before. This year he is planting soybeans. Explain why he probably did not increase his cowpea yield by inoculation and how his decision not to inoculate could affect this year's soybean crop.
4. A farmer inoculated her legume seed more than two weeks ago, but could not plant them when planned. What would you advise?
5. group of farmers has approached you about manufacturing inoculant in their village. They see the inoculant as a finely powdered soil and feel that they could benefit by manufacturing it locally. Describe the production process and explain what they will need to produce inoculant successfully.
6. Take three common crop systems in your area and develop a set of inoculation recommendations for farmers. Consider the entire system—climate, soil, management, species, and any other relevant factors. Recommend inoculation rates and methods, considering the availability of materials, local farming practices, and the education level of the farmers.

Questions

1. When should I inoculate?
2. How is inoculant different from fertilizer?
3. How can you tell that an inoculant is working after it has been applied?
4. Why do inoculant producers list expiration dates?
5. What do we mean when we say that inoculants are specific for particular legume crops?
6. Why can't I use one inoculant for all legumes?
7. What is the major benefit of growing legumes besides the grain or forage yield?
8. How can I tell a good inoculant from a bad one?
9. What is the best way to store inoculant?
10. When should I pellet seeds?
11. When should I inoculate the soil rather than the seeds?
12. What is the best way to inoculate trees?
13. Is seed treatment the same as seed inoculation?
14. Why shouldn't I inoculate seeds treated with pesticides?

SUGGESTED LESSON PLAN FOR MODULE 5

TIME: 1 day +

OBJECTIVES:

Knowing what inoculation is and how to select good inoculants. Understanding how inoculants are used in the field. Knowing how to apply inoculant to seed or field. Knowing how to care for inoculants.

MATERIALS:

Demonstrations D5/3, D5/5, D5/6, and D5/7

Training Aids for Module 5

Display of appropriate materials, i.e., inoculant and/or rhizobial culture; stickers; tools.

STEPS:

1. Assemble materials and make preparations for hands-on learning during demonstrations D5/5.

2. Put up display of training aids and other items.

3. Divide this lesson into short lectures separated by hands-on demonstrations.

4. You will have to decide how much information you will give on how inoculants are produced. Although how inoculants are produced is critical to the farmer getting a good product, you may want to limit this part of your lesson to where inoculants can be obtained.

5. Each participant should practice all three methods of inoculation since this is critical to technology transfer. Your assessment of the ability of participants to inoculate seeds will be your learning evaluation of this lesson.

KEY CONCEPTS

There are many soil conditions which make it necessary to inoculate legume crops to get maximum yields.

The choice of methods for seed and soil inoculation depends on materials available and climate and soil conditions.

The proper inoculant must be used with each legume.

Inoculant contains living organisms which must be protected from heat and sun.

If inoculant is not stored properly, the number of rhizobia in the inoculant will decline.

Poor inoculant quality is an important reason that farmers do not get yield increases from inoculation.

Inoculant production is a process which requires specialized equipment, knowledge and skills.

Various types of inoculants are produced for various needs.

Table 5-6. Fungicides known to be toxic to rhizobia.

Captan	N-trichloromethylthio-4 cyclohexane-1, 2 dicarboximide
Carboxin	5, 6 dihydro-2-methyl-N-phenyl-1,4 oxathiin-3 carboxamide
Chloramil	2,3.5,6 tetrachloro-1,4 benzoquinone
PCNB	Pentachloromitrobenzene
Thiabendazole	"Tecto" 2-(4'thiazolyl)-benzimidazole
Thiram	Tetramethyl-thiuram-disulphide

Table 5-7. Most commonly used Insecticides.

Carbofuran	2, 3-dihydro-2, 2-dimethyl-benzofuranyl methyl-carbamate
Phorate	0-0 diethyl S-(ethyltrio)-methyl phosphorodithiate
Aldicarb	2-methyl-(methylthio) propionaldehyde-0-(methyl-carbomoyl) oxime

Figure 5-9. Inoculant applicator. Liquid inoculant (inoculant mixed with water) can be applied before planting using this method.



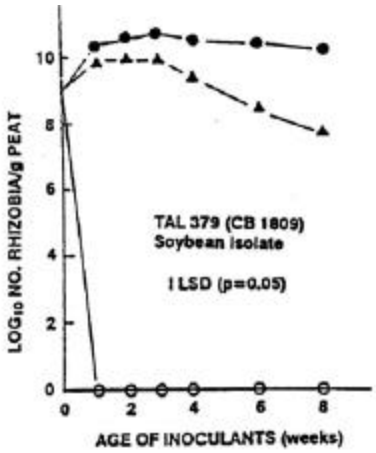


Figure 13. Survival of soybean rhizobia at three temperatures.

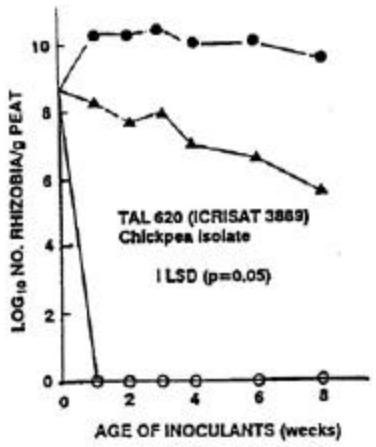


Figure 5-14. Survival of chickpea rhizobia at three temperatures.

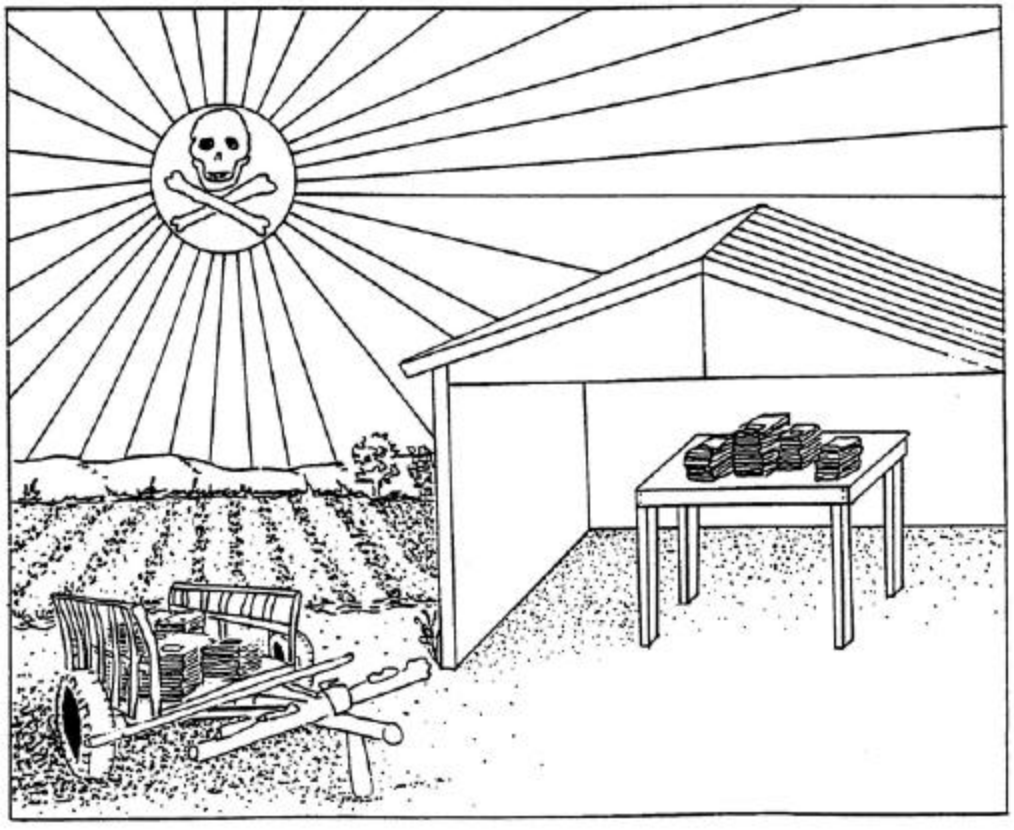


Figure 5-15. Inoculants should be transported to the field in a cool container. These farmers are carrying inoculant in a basket covered with a wet cloth.



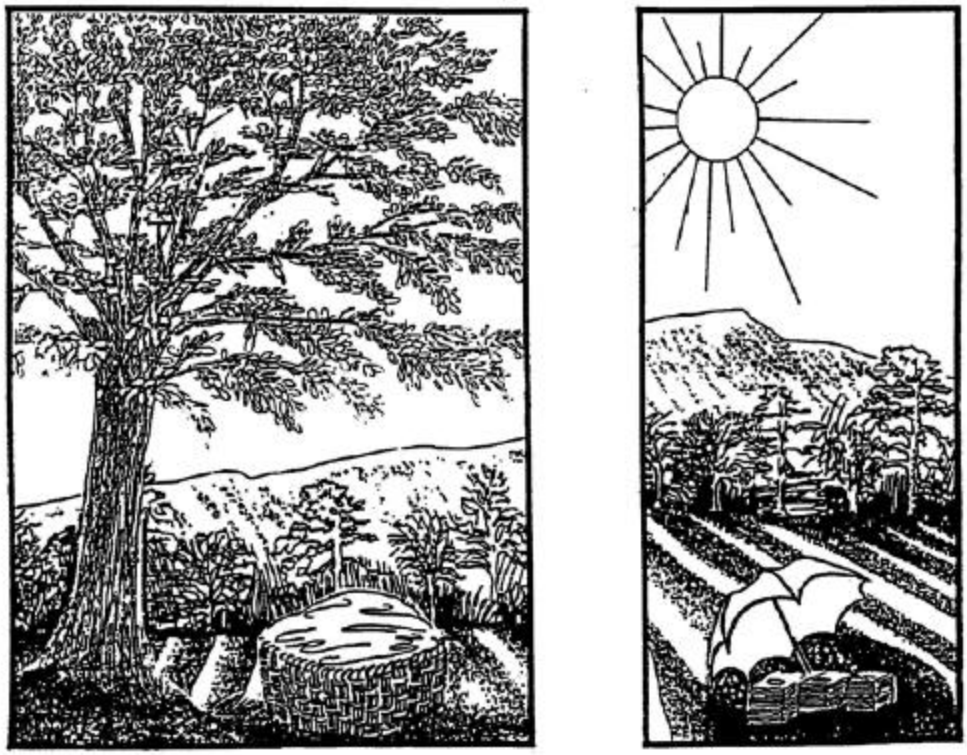


Figure 5-16. This inoculant is protected from the sun by an umbrella. Inoculant may also be placed under a shady tree.

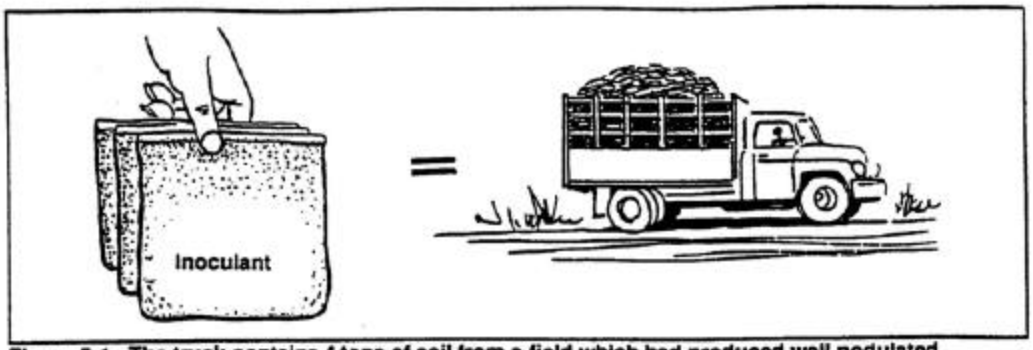


Figure 5-1. The truck contains 4 tons of soil from a field which had produced well nodulated legumes. The hand holds three 100 gram bags of rhizobial inoculant. There are the same number of living rhizobia in the three small bags as in the truck filled with soil.

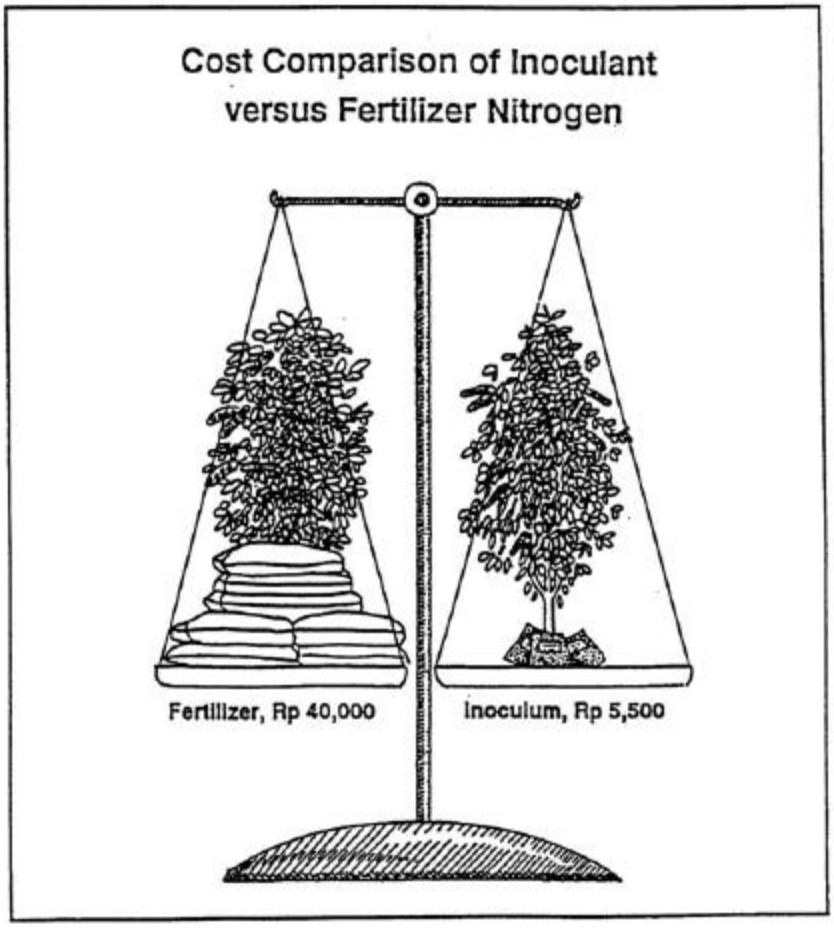


Fig Effect of different stickers on the number of viable inoculant rhizobia on soybean seeds at time of planting.

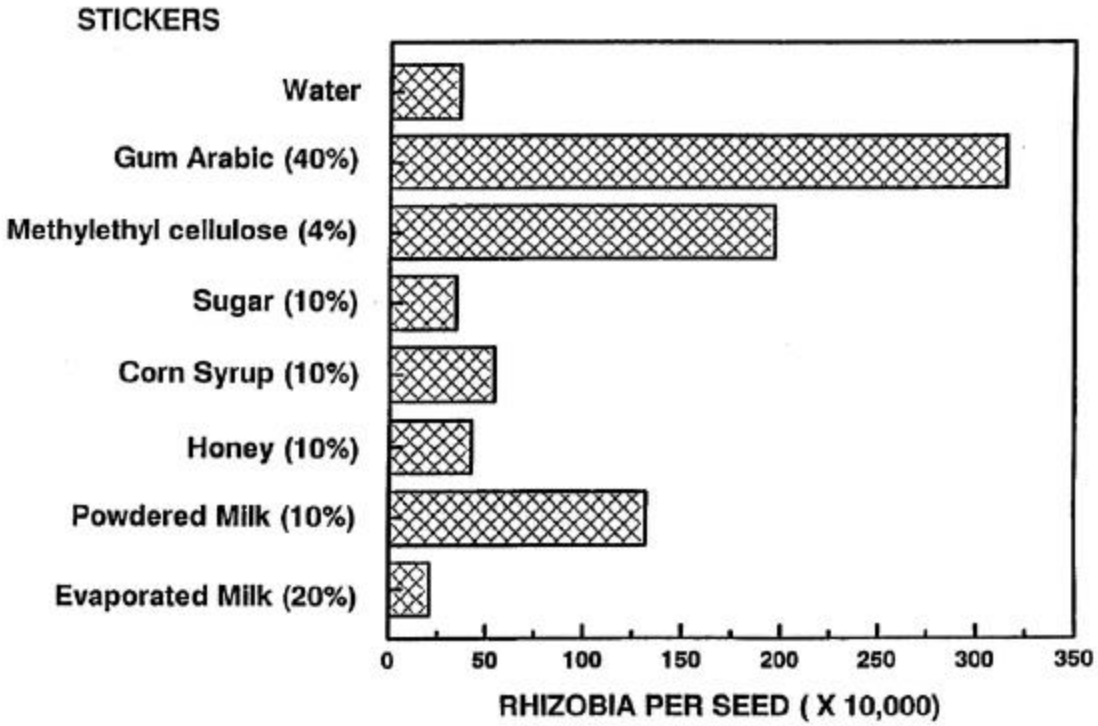
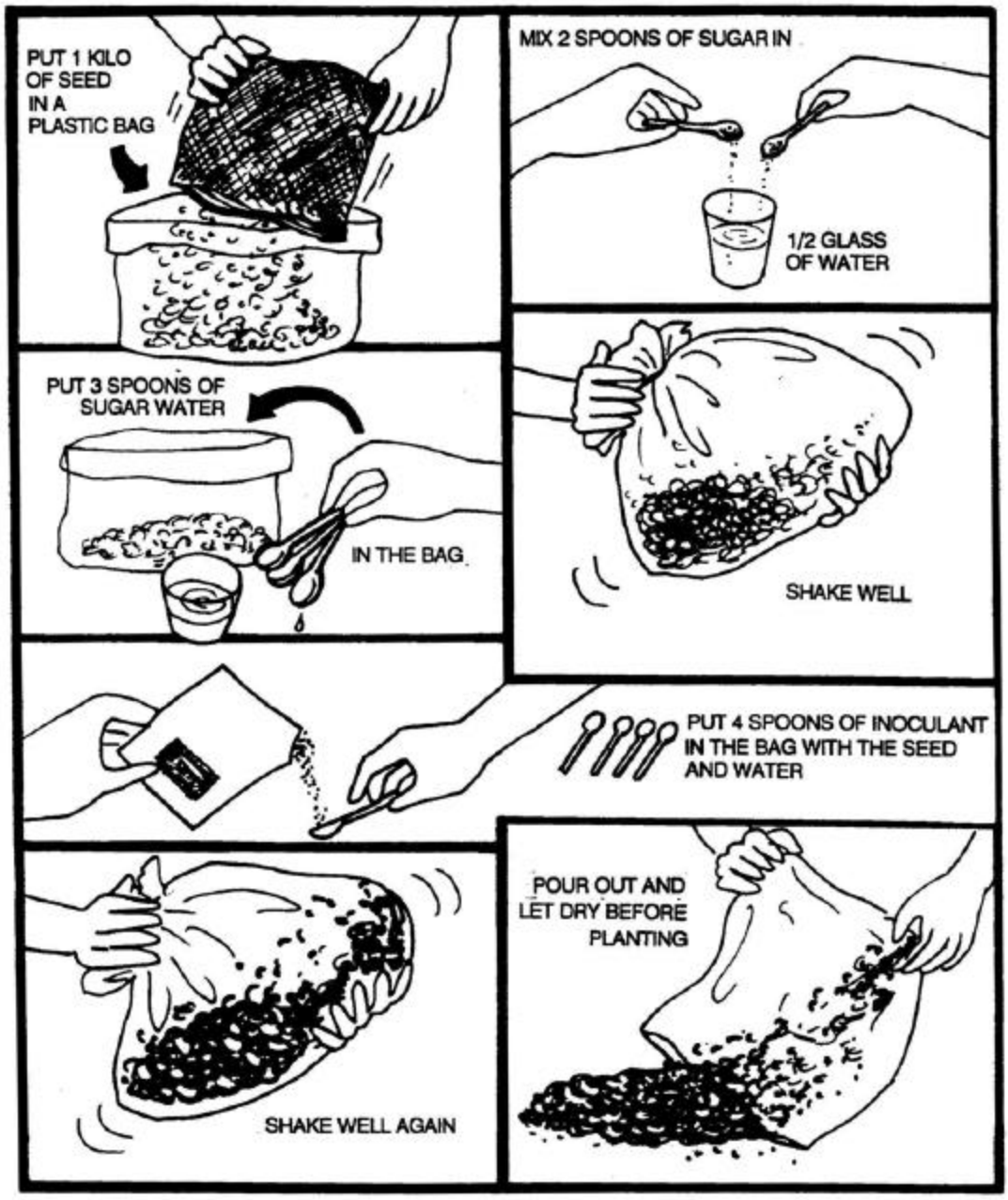




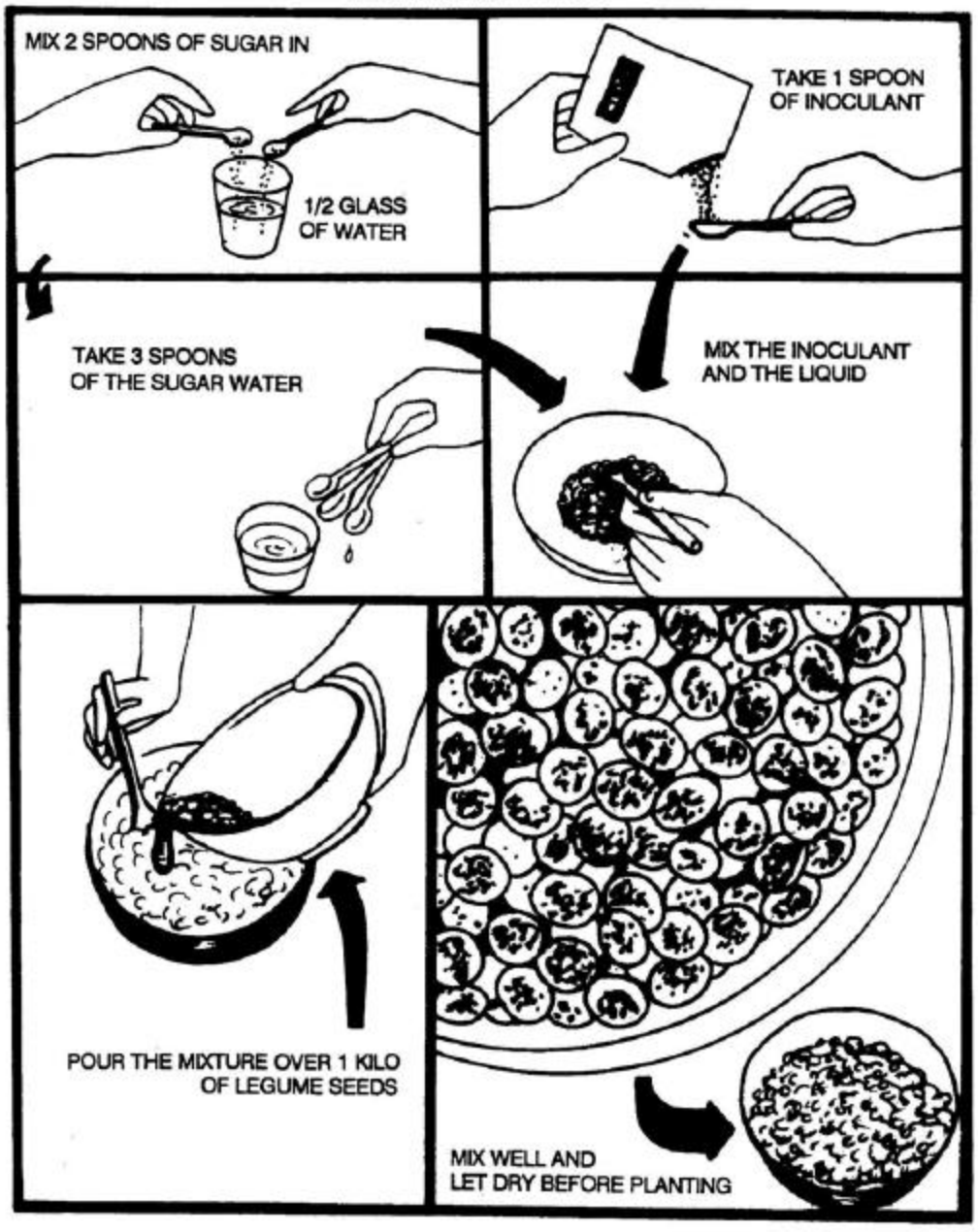


Figure 5-12. It is best to store inoculants in cool places. Refrigeration between 4 and 26 C is ideal. However, using a buried urn in a shady place will allow good storage for at least 6 months.

SHAKING METHOD



MIXING METHOD



MODULE NUMBER 6

RESPONSE TO LEGUME INOCULATION

SUMMARY

In previous modules we learned many facts about legume BNF. For example, we learned that: 1) different legume crops require different types of rhizobia; 2) legume plants must photosynthesize in order to fix nitrogen; and 3) inoculant quality is crucial if inoculation is to result in increased yields. In this module, we will use many of these earlier concepts to examine inoculation and legume BNF in farmers' field. A response to inoculation is any increase in yield, seed protein content, or other benefit to the farmer that is due to inoculation of a legume crop with rhizobia. The most important concept to remember from this module is the **Law of the Minimum**. This law states that the yield from a farmer's field is always limited by a single factor—yield will increase only when that factor is improved. For example, legume crops will increase with rhizobial inoculation only if the factor limiting yields is nitrogen. BNF cannot do its part in increasing crop production if yields are limited, for instance, by soil pH factors, low phosphorus, or disease or insect problems.

This module also will explain how the native rhizobia already in the soil can affect a legume crop's response to inoculation. Native rhizobia can prevent the rhizobia introduced in the inoculant from forming nodules on the crop. In other cases, the native rhizobia can fix as much nitrogen as the plant needs, making inoculation unnecessary.

KEY CONCEPTS

- Although most farmers think a response to inoculating their crops means yield increases, there are other important benefits from inoculation such as improved protein content of seed.

- Rhizobial inoculant can only improve farmers' yields when their legume crops do not have enough nitrogen. Inoculant will not solve other problems such as lack of other soil nutrients. This concept is called the **Law of the Minimum.**

- Inoculant and BNF improve yields best when proper farm management is practiced.

- Nitrogen already in the soil or left over from earlier fertilizer applications may reduce BNF and the benefit from inoculation.

- When there are already many rhizobia in the soil that can stimulate effective BNF, inoculation may not provide much further benefit.

BENEFITS FROM LEGUME INOCULATION

Higher Yields

When farmers purchase agricultural inputs, they expect to increase their yields. Legume inoculant is an input, and farmers expect to increase their legume yields when they inoculate. In fact, yield increases from inoculation can be large, but some legume crops at some sites may not increase their yields at all.

Table 6-1 shows the response to inoculation of four legume crops at two sites in the Philippines. At both sites, inoculation increased soybean yields considerably, indicating that farmers who grow soybean will find inoculation profitable. Yield increases were also good for common bean at the Ilocos site. For the other crops, inoculation did not increase yields substantially.

Table 6-1. Seed yields (kg/ha) from four legume crops at two sites in the Philippines with and without inoculation.

	Ilocos		Camarines Sur	
Legume	**Inoc**	**No Inoc**	**Inoc**	**No Inoc**
Soybean	2200	1620	2189	1683
Common Bean	3280	2410	369	316
Mungbean	775	665	526	302
Groundnut	1250	1325	907	737

Several points are worth noting. For one thing, there were large differences in yields between the sites. For example, yield of common bean at Ilocos was almost ten times the yield at Camarines Sur. There were also large differences in yields between different crops at the same site. These observations indicate the large effects that species, climate, and soil can have on crop yields.

The results in this table show clearly that inoculation will not increase yields of every legume crop at every site. This module will help explain how the response to inoculation can vary so widely. If you understand how legumes respond to inoculation, then you will know how to evaluate the results of inoculation and you will be able to make good recommendations to farmers.

Higher Protein Content

Farmers are most interested in yield increases, but these are not the only potential benefits from inoculation. It is important to understand the other benefits, even if they are not easy to detect or measure. Inoculation can increase the protein content of seed even if there is no increase in yield.

One of the main reasons we grow legumes is for the protein content in the seed. Nitrogen is a key component of this protein. For example, soybean seed may have up to 6.5% nitrogen (40.6% protein) and mungbean up to 3.8% nitrogen (23.8% protein). The protein content of cereal crops is much lower. For example, maize seed may have only 2.2% nitrogen.

Table 6-2 shows that inoculation increases the protein content of seed even when it does not increase yield. Although increases in nitrogen content appear to be small, each 1% increase in nitrogen means a 6.25% increase in protein.

Table 6-2. Increases in legume seed yield and nitrogen content due to rhizobial inoculation.

	Number of Trials Where Inoculation Increased		Average Seed Nitrogen (%)	
Legume Species	**Yield**	**Seed Nitrogen**	**Inoculated**	**Uninoculated**
Soybean	83	100	6.2	5.7
Lima bean	60	80	3.1	3.0
Common bean	33	50	3.0	2.8
Cowpea	0	80	4.2	3.9

Source: J. Thies, Ph.D. thesis, University of Hawaii, 1990.

How does inoculation increase seed protein content even when it does not increase yields? The answer stems from the fact that legume plants produce as many seeds as they can. When available nitrogen is low, the plant reduces the protein content of each seed in order to produce the same number of seeds with a limited amount of nitrogen. By increasing the available nitrogen, inoculation allows the plant to produce seeds with a high protein content.

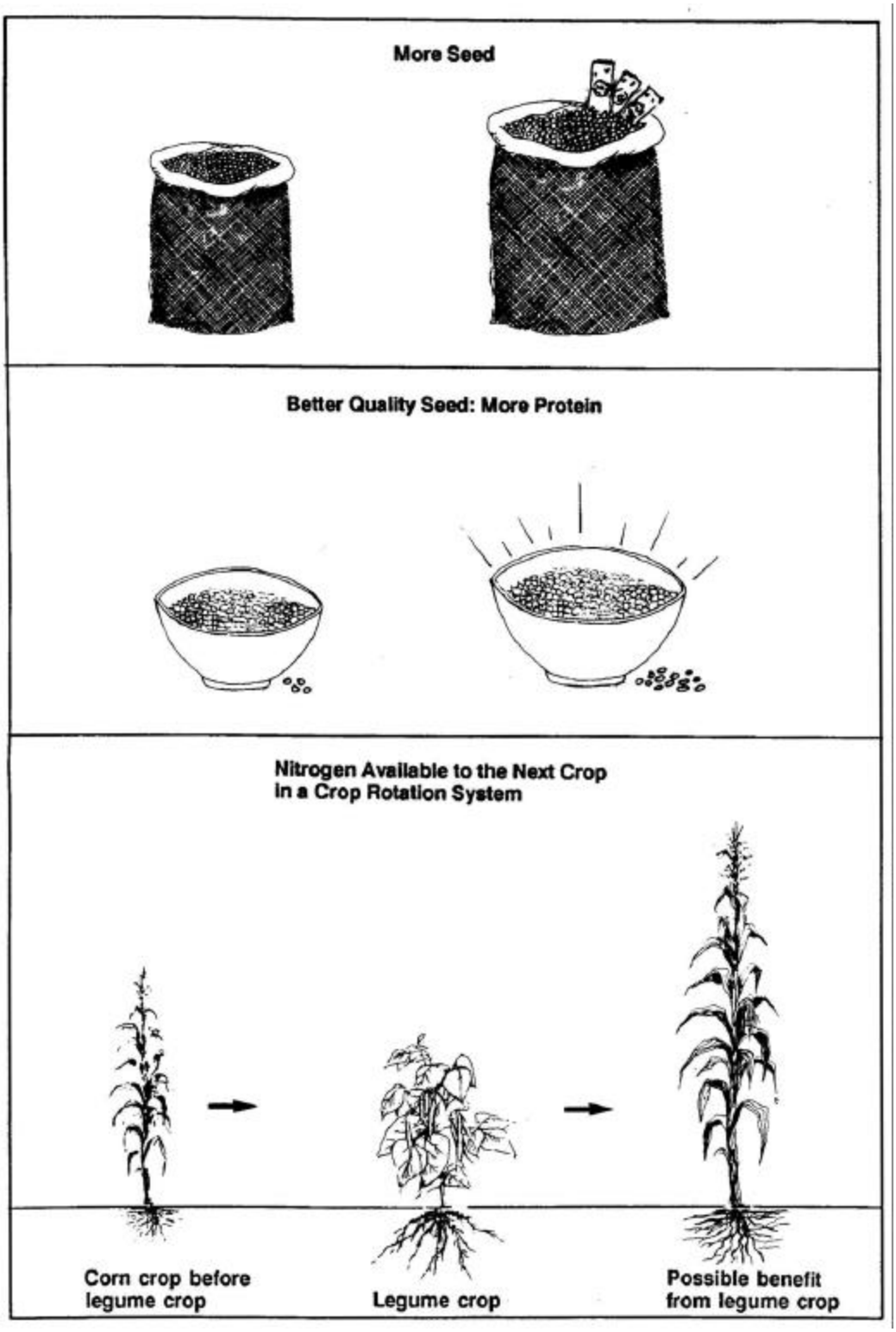
More Soil Nitrogen Available for Other Crops

With increased nodulation, a legume crop can obtain more nitrogen from BNF to support higher growth and nitrogen content. If crop residues are returned to the soil, more nitrogen is available for the next crop. Although difficult to measure, this benefit from inoculation may add to a farmer's income in the long term.

FACTORS AFFECTING THE SUCCESS OF LEGUME INOCULATION

As shown in **Table 6-1**, a yield response to inoculation is not always guaranteed.

Figure 6-1. Farmers may realize increased yields from legume inoculation. There may be other less visible benefits from inoculation. Whether other benefits are realized by farmers results from soil, climate, and management conditions.



However, **Table 6-3** shows that measurable yield increases from inoculation are common for many tropical legumes.

Table 6-3. Percentage of NifTAL international trials in which rhizobial inoculation increased yields.

Legume	Number of Trials	Trials Where Yields Increased (%)
Groundnut	26	50
Soybean	36	64
Mungbean	40	53
Leucaena	8	38
Common Bean	10	10
Cowpea	9	56

Source: NifTAL International Legume Inoculation Trials.

For the results reported in **Table 6-3**, a positive response to inoculation was defined as a yield increase of more than 1.0 standard deviation. This means an increase of about 150 kg/ha, based on an average yield of 1000 kg/ha and a coefficient of variation of 15%. Even at sites where yield increases did not meet these statistical standards, the actual increases were large. In general, inoculation at these sites will be profitable for farmers.

How can we explain why some inoculation trials show a response to legume inoculation and others do not? Clearly, if we could tell farmers exactly which crops to inoculate, they could invest their money in inoculant wisely. Techniques are being developed to predict whether legume inoculation will increase yields, but there is still no easy way for extension agents to tell farmers what sort of increases they can expect. However, if you understand how management and environmental factors affect the BNF process, you will be able to help farmers increase their yields where possible and make wise decisions about inoculating their crops.

Is the Inoculant Alright?

We learned in **Module 5** that the quality of legume inoculant is determined by the number of live rhizobia in the inoculant and their effectiveness in stimulating BNF. One reason for low yields may be the use of the wrong inoculant. Check the cross-inoculation groups listed in **Module 3** to make sure that the inoculant you recommend is suitable for the legume crop the farmer is planting.

Poor inoculant quality is often the reason for low yields. For example, if farmers are to obtain the highest possible soybean yields, the inoculant must contain at least one million (1×10^6) live rhizobia per seed. If rhizobia numbers are low, the farmer can compensate to

some extent by applying more inoculant per seed. However, if the number of live rhizobia falls below 1 million per gram of carrier, the farmer cannot apply enough inoculant to obtain maximum yields.

Table 6-4. Inoculant quality affects the yields of legumes.

Rhizobia in Inoculant	Rhizobia per Seed	Seed Yield (kg/ha)
0/g peat	0	1502
$3x10^5$/g peat	$2x10^2$	1876
$3x10^7$/g peat	$2x10^4$	2143
$3x10^9$/g peat	$2x10^6$	3217

Source: R. Nyemba, M.Sc. Thesis, University of Hawaii.

The way the farmer stores and handles inoculant to keep the rhizobia alive is very important. Old inoculant or inoculant that has been badly stored should not be used. Inoculant or coated seed should not be exposed to heat or sunlight. Cool soil temperatures with good moisture supply keep rhizobia alive until they make contact with the root at seed germination. **Providing farmers with good inoculant and teaching them correct application methods—these are the most important steps to improve BNF in the field.**

Is BNF Really What the Crop Needs?

Some principles of nature are so important that they are called laws. One law that has important implications for agriculture is the **Law of the Minimum**. **Figure 6-2** demonstrates this concept. It shows two barrels made of wooden staves. The height of each stave represents the amount of a particular nutrient or other factor available for plant growth. The water will always run out at the lowest stave, no matter how high the others are.

Similarly, a plant will always stop growing when it runs out of a key nutrient or other requirement for growth. In the first barrel (a) the shortest stave is phosphorus. No matter how high the level of other nutrients and growth requirements (the other staves), crop production cannot increase above the level possible with this amount of phosphorus. The factor in the smallest supply (the shortest stave) will determines the size of the farmer's yield. This is called the **limiting factor**. Adding more of the other factors (inoculant to stimulate nitrogen production, for example, or water) will not increase plant growth. In this case, farmers will only obtain higher yields from inoculation if they also increase the amount of phosphorus (the limiting factor) available to their crops.

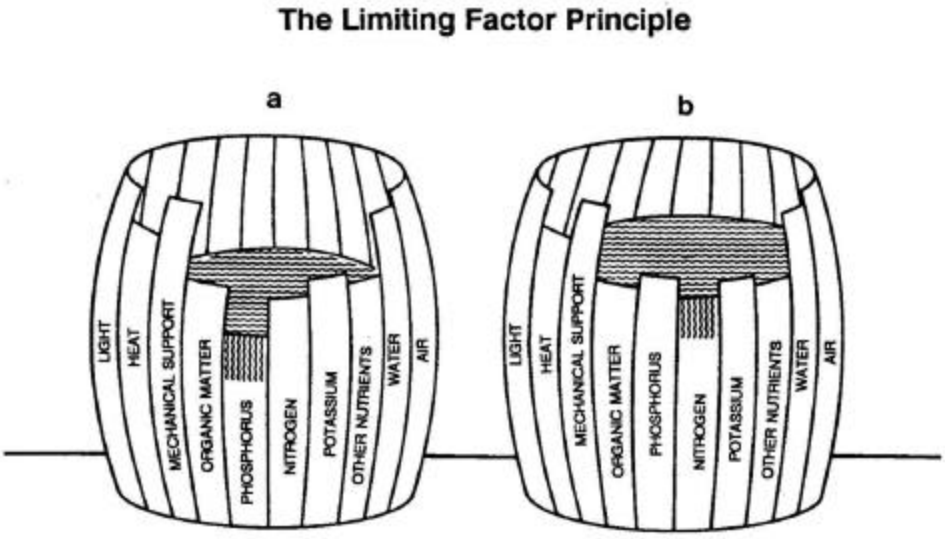


Figure 6-2. Nutrient limitations are important considerations in The Law of the Minimum.

The second barrel (b), with its higher water level, illustrates how yields are raised when the limiting factor is increased. In this case, the farmers add phosphorus and the phosphorus stave becomes longer. Now nitrogen becomes the limiting factor (the shortest stave). The farmers can increase their legume yields again by inoculating their crops or adding nitrogen fertilizer. When legume yields increase in response to inoculation, the Law of the Minimum tells us that nitrogen must have been the limiting factor affecting crop growth.

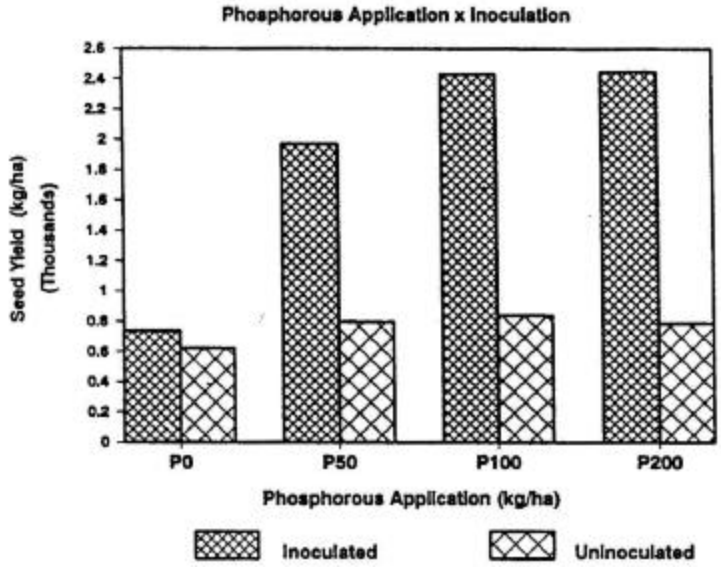


Figure 6-3. Phosphorus deficient soil limits response to inoculation.

Figure 6-3 demonstrates how the Law of the Minimum works when more than one nutrient is limiting plant growth, a situation often encountered in farmers' fields. The figure shows results of an inoculation trial with soybean conducted in soil that was low in phosphorus. Four rates of phosphorus fertilizer were added to both inoculated and uninoculated plants.

Phosphorus is the first limiting factor for this crop: When no phosphorus is added (0), there is little or no yield increase with inoculation. When phosphorus is added, nitrogen becomes the limiting factor: Adding phosphorus alone increases yields very little. Additions of phosphorus **plus** inoculation result in large yield increases, and the response to inoculation increases as more phosphorus is applied. By remembering the Law of the Minimum, you should be able to explain why the response to inoculation increases when more phosphorus fertilizer is added to the soil.

Remember too that the Law of the Minimum applies to **all** factors that affect crop growth, not just soil nutrients. If a legume crop is limited by a factor such as water or low soil pH, the plants will not form many nodules or fix much nitrogen even with the addition of rhizobial inoculant, and yields will not increase. As a general rule, farmers will obtain greater benefits from inoculation when they take care of other factors limiting crop growth through good management practices. As an extension agent, you must identify the specific factors limiting crop growth in your area so that you can advise farmers on how to invest in crop inputs and improved management.

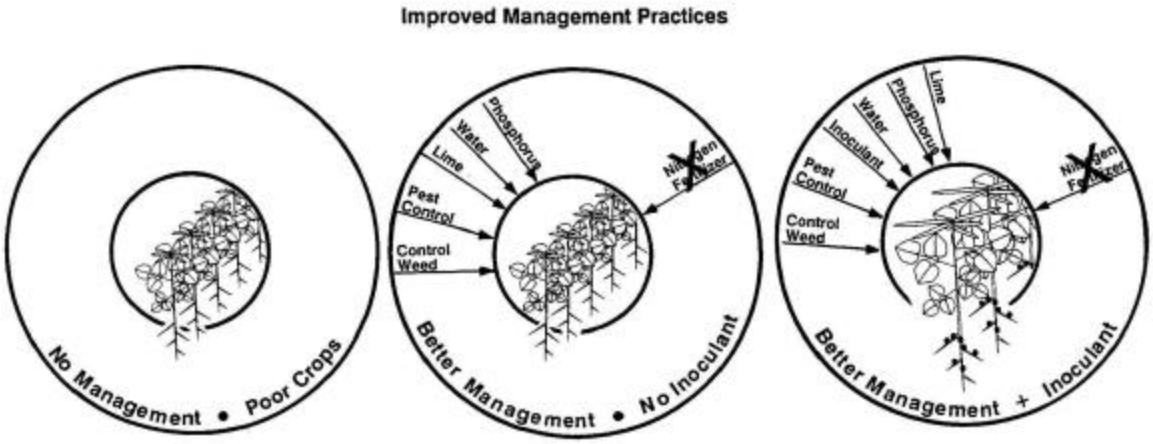


Figure 6-4. Good management practices ensure good crops and benefits from legume inoculation.

Table 6-5. Amount of nitrogen in some common crops.

Crop	Seed Yield	Nitrogen	
		Seed	Total
	- - - - - - - - - - - - - - kg/ha - - - - - - - - - - - - - -		
Maize	2000	31	36
Rice	3000	36	42
Cassava	100000	19	22
Soybean	2000	121	143
Mungbean	1000	38	25
Cowpea	1200	48	56

INOCULATION AND NITROGEN FERTILIZER

Although legumes can fix atmospheric nitrogen through BNF, they also use nitrogen in mineral form (NO_3 and NH_4). Mineral nitrogen in a farmer's field may come from the soil (mineralization of organic matter) or from fertilizer, manure, or residual nitrogen from a previous crop. In fact, legumes prefer to use nitrogen from the soil as this requires less energy than making their own nitrogen through BNF. If there is already enough mineral nitrogen in the soil, there will be no benefit from inoculating the legume crop. However, if it's a question of adding nitrogen, BNF is generally a better option than fertilizer. There are a number of reasons for this.

For one thing, legumes are rich in protein, with a high nitrogen content. They thus have higher requirements for nitrogen than cereals or root crops. Farmers would have to apply large amounts of fertilizer to meet all nitrogen needs of their legume crops. In addition, legumes use the nitrogen produced through BNF much more efficiently that they use nitrogen applied as fertilizer. To stimulate good growth, farmers need to apply two to three times more nitrogen fertilizer than the legume crop actually requires. A farmer would have to apply 621 to 933 kg of urea per hectare (equivalent to 286 to 429 kg nitrogen) to obtain the same soybean yield (2000 kg/ha) that would be possible with BNF and no nitrogen fertilizer.

In general, it is much more efficient and less expensive to produce legumes with BNF than with nitrogen fertilizer. However, many farmers apply a small amount of nitrogen, called **starter nitrogen**, to their legume crops at planting. This is because it takes several days for inoculated rhizobia to infect the root, form nodules, and begin BNF. Until BNF takes effect, the legume needs nitrogen from the soil.

Table 6-6. Response of soybean and common bean to starter nitrogen.

	Soybean		Common bean	
N Applied	**+ Inoc**	**- Inoc**	**+ Inoc**	**- Inoc**
kg N/ha	- - - - - - - - - - - - - - - kg seed/ha - - - - - - - - - - - - - - -			
0	2160	1340	2650	1540
10	2250	1640	2630	1760
30	2370	1580	2910	2130
60	2200	1620	3280	2410

Source: Unpublished data from Ilocos Norte, Philippines by Singleton, Escano, Layaoen.

All legumes grow better and fix more nitrogen if some soil nitrogen is available before the nodules form and BNF begins. If there is at least some nitrogen in the soil, seedlings will be larger when the first nodules are formed and more photosynthetic energy will be available for nodule development.

Should the farmer apply starter nitrogen at planting? The answer depends on several factors such as legume species, soil type, climate, and the amount of nitrogen already in the soil. **Table 6-6** shows how two legumes, soybean and common bean, re-sponded to inoculation and four levels of starter nitrogen. Both crops had much higher yields with inoculation, indicating that there was not enough soil nitrogen or native rhizobia to meet their nitrogen needs. The inoculated soybean did not benefit sig-nificantly from the starter nitrogen, but the inoculated common bean did. Uninoculated crops responded to starter nitrogen, but the response was much smaller for soybean.

Not only do legume species respond differently to inoculation, but the potential benefits from starter nitrogen also depend on soil and weather conditions. For example, leaching of the starter nitrogen is a problem in well-drained soils where rainfall is high. Small plants with small root systems cannot intercept starter nitrogen. In general, starter nitrogen will increase yields only in soils that are **extremely deficient in nitrogen**, and where crop yield potential is high. Starter nitrogen should only be recommended to farmers if there is convincing evidence that there will be an economic benefit. In addition, **starter nitrogen should only be recommended for crops that are also inoculated**.

INOCULATION AND NATIVE RHIZOBIA

Native rhizobia are present in many soils, depending on the presence of wild legumes, a history of previous legume crops, and factors such as soil pH and rainfall. Numbers of native rhizobia can range from none to many millions. The size of rhizobial populations in the soil is an important factor affecting the response to legume inoculation (**Table 6-7**).

Table 6-7. Effect of native rhizobia on inoculation success.

Number of Rhizobia	Nodules Formed by Inoculant
number/g soil	%
11	71
11	53
1318	34
5495	38
93325	7
229086	12

Source: Weaver and Frederick, 1974. Effect of inoculant rate on competitive nodulation of *Glycine max*. Agron J. 66:233-236.

Extension agents and farmers cannot easily measure populations of native rhizobia in the soil. However, an understanding of the conditions that favor large rhizobial populations allows the extension agent to assess whether native populations are likely to affect crop responses to inoculation. Generally, the number of rhizobia in the soil depends on the number of legume plants growing in the field and the number of times legumes have been cropped in the past.

Sites with dry climates have few rhizobia in the soil, while sites with higher rainfall have more vegetation and legumes and therefore more native rhizobia. At the other extreme, some climates with extremely high rainfall have acid, infertile soils. Legumes often do not grow well in these soils and thus rhizobial populations are low.

The particular species of rhizobia found in a soil depends on the species of legumes growing at the site. Many tropical soils contain rhizobia for a wide range of legumes. These native rhizobia may stimulate nodulation in cowpea and peanut because these legumes cross-inoculate with many other tropical species. By contrast, soybean does not cross-inoculate with any wild legumes. There are usually no soybean rhizobia (*Bradyrhizobium japonicum*) in the soil unless soybean crops have grown there before. You may wish to refer back to the cross-inoculation groups listed in **Module 3**.

The presence of large numbers of native rhizobia can actually interfere with BNF. The native rhizobia may form nodules on the legume without going on to stimulate BNF themselves, but at the same time blocking nodule formation and BNF by the inoculated rhizobia. On the other hand, many populations of native rhizobia can stimulate enough BNF to meet a crop's nitrogen requirement. Where this is the case, inoculation will not produce any further increases in yields. Even if the introduced rhizobia form most of the nodules on the crop, there may still be no response to inoculation if the population of native rhizobia is large.

Table 6-8. The effect of numbers of rhizobia in the soil on the yield response to inoculation.

Country	Crop	Rhizobia no/g soil	Yield kg/ha	
			+ Inoc	- Inoc
Ecuador	Common bean	0	490	460
Ecuador	Leucaena	0	8215	6427
Morocco	Soybean	0	685	235
Hawaii	Soybean	0	3200	850
Philippines	Common bean	3	3280	2410
Hawaii	Groundnut	5	5800	4800
Taiwan	Soybean	23	1444	1179
Philippines	Mungbean	243	775	665
Morocco	Vicia sativa	1038	1875	1900
India	Groundnut	3546	2188	2059
Hawaii	Cowpea	35900	2850	2900

Source: Collaborators in the Worldwide Rhizobium Ecology Network (WREN).

Remember that inoculated rhizobia are only present on the seed coat or in the spot where soil inoculant has been added, whereas native rhizobia are present through the soil, with many opportunities to come into contact with crop roots. For inoculation to compete effectively with native rhizobia, the inoculant must contain very large numbers of live rhizobia—1000 times the number of native rhizobia per gram of soil.

Table 6-8 shows how the number of native rhizobia in the soil affects the yield response to inoculation. In these trials, there was little response to inoculation when there were more than 100 native rhizobia per gram of soil. Even when native rhizobial populations were fewer than 100/g soil, the response to inoculation was sometimes small. For example, common bean had a very small response to inoculation in Ecuador even though there were no native rhizobia at the site. Also, the response to soybean inoculation in Hawaii was much larger than in Morocco even though there were no soybean rhizobia at either site. Remembering the Law of the Minimum, could it be that the low yield responses to inoculation in Morocco and Ecuador were due to other limiting factors rather than nitrogen?

REVIEW, DISCUSSION, CASE STUDIES

The previous modules presented basic information about rhizobia, legumes, BNF, and legume inoculation. This module described how environmental and management factors influence the response to legume inoculation. With this information, you should now be better prepared to identify and solve problems related to legume BNF in farmers' field. The following are `case studies' that ask you to evaluate various problems and then give a solution. There is not necessarily just one correct answer. In fact, you may have to make several recommendations to solve a BNF problem or develop a viable BNF program.

To suggest good solutions, you must consider all the factors that influence BNF. The most important aspect of this exercise is to **first identify the problem** before thinking of a solution.

1. The Ministry of Agriculture has targeted a savanna region for increased oil seed production. The region under consideration is currently dominated by farmers with small holdings using land for shifting cultivation and grazing. The region has deep, well-drained Alfisols, rainfall of 1200 mm over a three- to four-month cropping season, and a soil pH of 6.5. A previous evaluation suggested that peanut performs well. Design an applied research program to identify whether inoculation is required at initial planting and in subsequent years under a maize/peanut rotation. Results of this research program will be used to plan and develop an inoculant production facility.

2. One farmer in your area has experienced problems with nodulation, while other farmers have not. Make a list of questions to ask this farmer to help determine what might be causing the problem. Give the possible answers to these questions and design a simple test to identify the particular aspect of BNF that needs to be addressed.

3. Several farmers that grew lima bean (*Phaseolus lunatus*) successfully are now experiencing nodulation failure when planting common bean (*Phaseolus vulgaris*). The extension service introduced inoculation technology when lima bean was introduced years ago and there was never nodulation failure on that species. What is likely to be the problem? What is the simplest way to identify the problem?

4. The Agricultural Development Board has designed an irrigation scheme in an arid environment (less than 150 mm annual rainfall) and another development scheme in an upland rainfed region (annual rainfall of more than 1500 mm). Formerly the upland area was in pasture. The Board wants you to introduce several different legume crops in the two areas—soybean, lima bean, cowpea, and common bean. They want you to make a preliminary evaluation of the need for inoculants without extensive field testing (funds are limited for research). What can you do during the next six months to assess inoculation needs in these two areas?

5. Farmers in a rice scheme rotate paddy with mungbean. They have adequate moisture for rice and apply significant fertilizer inputs including nitrogen, which is subsidized by the government. Mungbean is broadcast into rice before harvest at a density of 200,000/ha. Rainfall ends two weeks before the mungbean flowers. The

farmers inoculate their mungbean crops, yet nodulation is poor (low numbers of small nodules). Yields average 700 kg/ha. This system has been practiced for 100 years. The extension service has asked you to find out how to improve mungbean yields in this system through BNF technology. Can you help them?

6. There are many small inoculation producers in your country supplying inoculants to smallholder farmers growing traditional crops. The farmers' legumes are effectively nodulated, and there have been no complaints about the inoculants. The Ministry of Agriculture would like to place some controls on the inoculant industry following a successful program that ensured quality control of fertilizers delivered to farmers. You have been asked to determine whether controls are needed and to make recommendations for a program to ensure the production of high-quality inoculants. How will you go about this?

7. Soybeans are to be introduced in a large production scheme in the humid lowlands. Soils have been recently cleared from forest and are highly weathered. As team leader, you need to design a management package for a cropping system that can sustain productivity over time. Does BNF have a role, and what potential constraints to BNF need to be addressed?

SUGGESTED LESSON PLAN FOR MODULE 6

TIME: 2-3 hours +

OBJECTIVES:

Understanding that there are many things which affect the response of legumes to inoculation. Knowing what these things are and knowing how to overcome the problems they create. Knowing that the concept of The Law of The Minimum is important to calculating the benefits of legume inoculation.

MATERIALS:

Demonstration 06/1

Training Aids for Module 6

STEPS:

1. Set up field experiment if possible (07/1). This is very important for successfully presenting this module. Display key concepts and other training aids.

2. The material in this module is largely theoretical, yet the practical application of the information is the difference between successful and unsuccessful BNF transfer. Therefore, your learning will be challenged in this module and a thorough review of the resource materials will be necessary.

3. Much of the teaching can be done in the field during observation of the different treatment and results.

4. Again, use questions to evaluate learning and the potential of participants to continue the process of technology transfer with farmers.

KEY CONCEPTS

Although most farmers think a response to Inoculating their crops means yield Increases, there are other important benefits to Inoculation such as improved protein content of seed or Improved nodulation which means more BNF.

Rhizobia inoculant can only improve farmers' yields when their legume crops do not have enough nitrogen to meet the crop's requirements for growth. Inoculant will not solve other problems such as low soil fertility. This concept is The Law of the Minimum.

Inoculant and BNF improve farmers' yields best when proper farm management is practiced.

Nitrogen In the soil or left from fertilizer applied to previous cereal crops may reduce BNF and the benefit from inoculation.

When there are many rhizobia already in the soil that are very good at BNF with the farmer's crop, the farmer may not have a large benefit from inoculation.

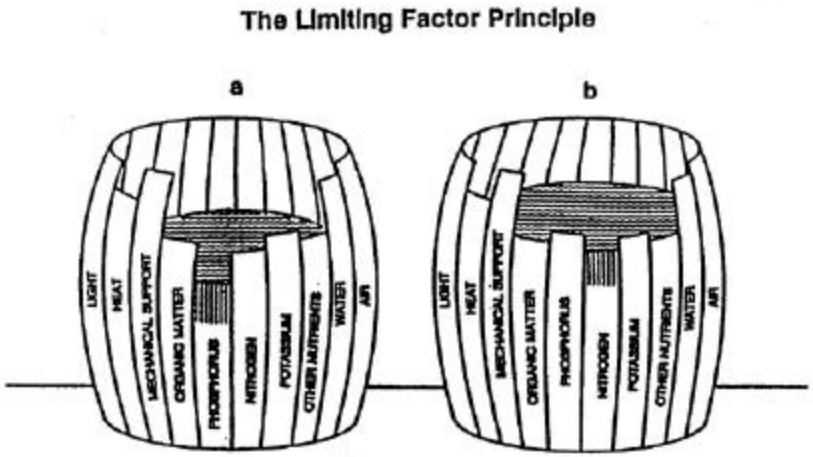
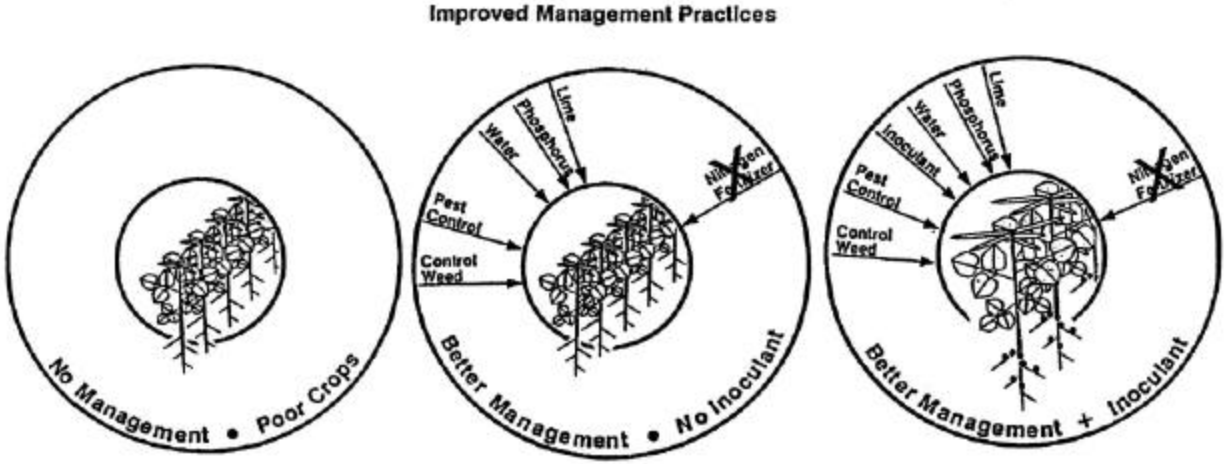
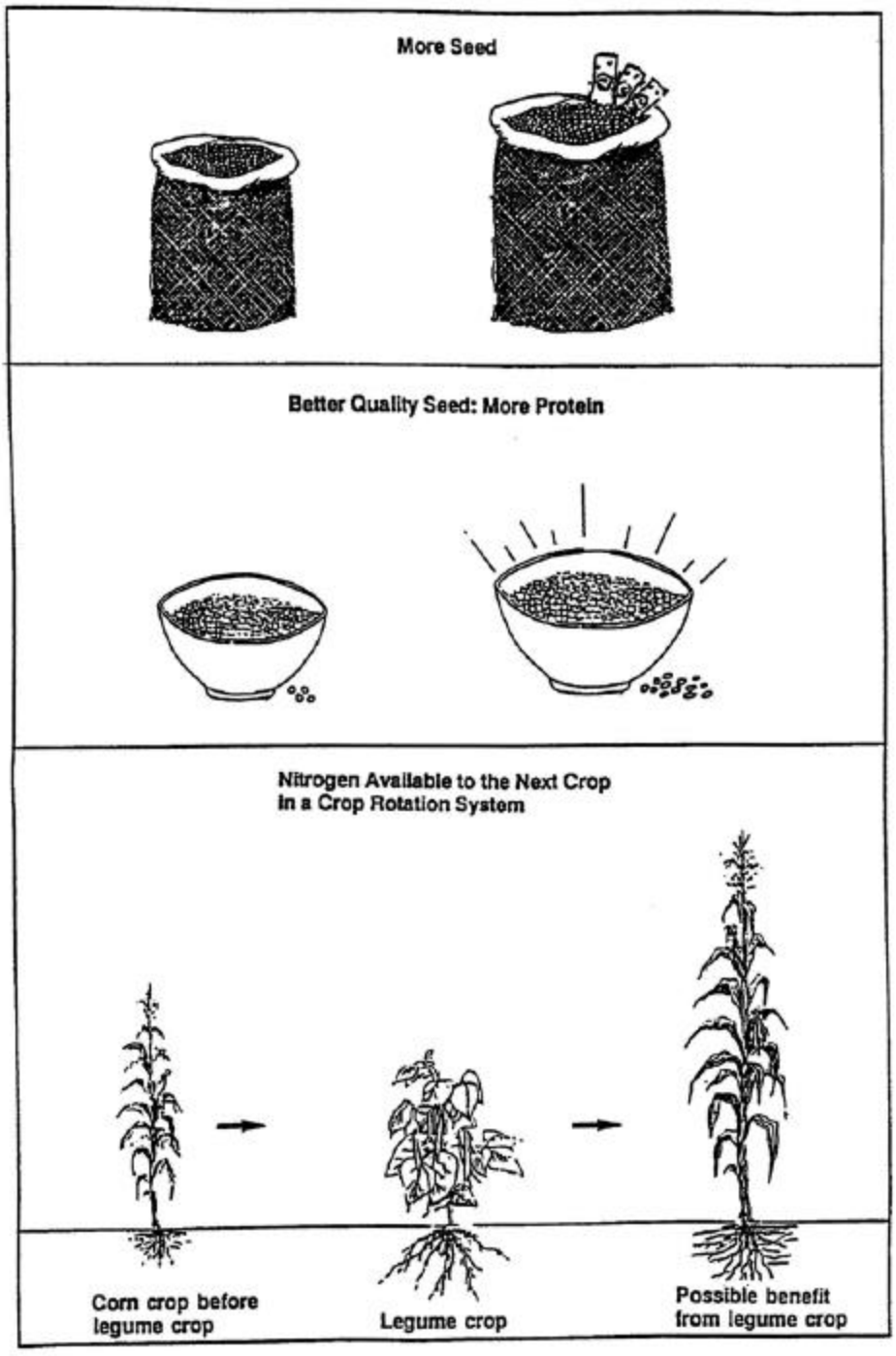


Figure 6-2. Environmental factors and nutrient limitations are important considerations in the Law of the Minimum.




More Seed
Better Quality Seed: More Protein
Nitrogen Available to the Next Crop
in a Crop Rotation System
Corn crop before
legume crop
Legume crop
Possible benefit
from legume crop

MODULE NUMBER 7

TESTING AND EVALUATING BNF IN THE FIELD

SUMMARY

Previous Modules discussed the nature of legume BNF, how farmers benefit from inoculation, methods of inoculating legumes, and effects of the environment on BNF and the response to inoculation. Understanding the principles of earlier modules is important for the extension agent to evaluate the success or failure of BNF in the field, and to make appropriate recommendations to farmers. This Module presents information to help the extension agent correctly identify problems with BNF in the field. Diagnostic methods are presented which help the agent interpret their observations and formulate proper solutions to problems. Methods to measure the response to inoculation are presented. These methods will help the extension agent design appropriate tests and experimental programs for determining whether farmers will benefit from inoculation. A discussion on the economics of inoculation and benefits to farmer income is provided in this Module.

KEY CONCEPTS

- Training extension workers in applied BNF technology can help farmers make appropriate decisions about inoculating legume crops.
- There is a logical process that leads to appropriate farmer recommendations to inoculate:
    1. identifying problems with BNF in the field
    2. designing appropriate tests to validate the value of inoculation
    3. economic interpretation
    4. training and extension work
    5. recommendation to farmers to inoculate
- **Recommendation domains** are groups of farmers who are likely to benefit from inoculation technology in a similar way. Farmers belong to a recommendation domain when conditions on their farms are similar.
- Inoculation is an inexpensive technology; the risk of monetary loss to the farmers is low and the potential gain is very high.
- Analysis of on-farm trials to test the response to inoculation requires special but simple approaches.
- There are many ways to test the crop response to inoculation, including experiment station field experiments, greenhouse pot tests, soil surveys, and on-farm trials. Each has specific advantages.
- Non parametric statistics are an appropriate method to evaluate the response to inoculation in on-farm trials.
- Economic analysis of inoculation technology compares the cost of inoculation to the

increased revenue the farmer gets from inoculation.

IDENTIFYING THE NEED FOR INOCULATION AND INTERPRETATION OF DATA

We recommend the inoculation of all nitrogen-fixing legume crops, because the cost of inoculation is small, and the potential benefits are large. Even when the farmer does not see measurable increases in yield, he may benefit from increased seed protein and improved N status of his soil due to inoculation.

However, if farmers are to accept inoculation of legume crops as a standard practice, they must be assured of benefit from the inoculation. Ultimately, they are most interested in the economic benefits. It is therefore important to correctly identify the need for inoculation when promoting BNF technology.

This module discusses some methods that can be used to identify and test problems related to legume BNF on the farm, and measure the benefit that farmers can expect from inoculation.

Farmers Benefit From Inoculation Technology Only When Lack of Nitrogen Limits the Yield of Their Legume Crop: Review of a Basic Principle

In **Module 6** we learned that a legume crop can only benefit from inoculation if there was not enough nitrogen from other sources to support the growth of the crop. The other sources of nitrogen were identified as mineral N from the soil and fertilizer, and BNF from the native rhizobia already in the soil.

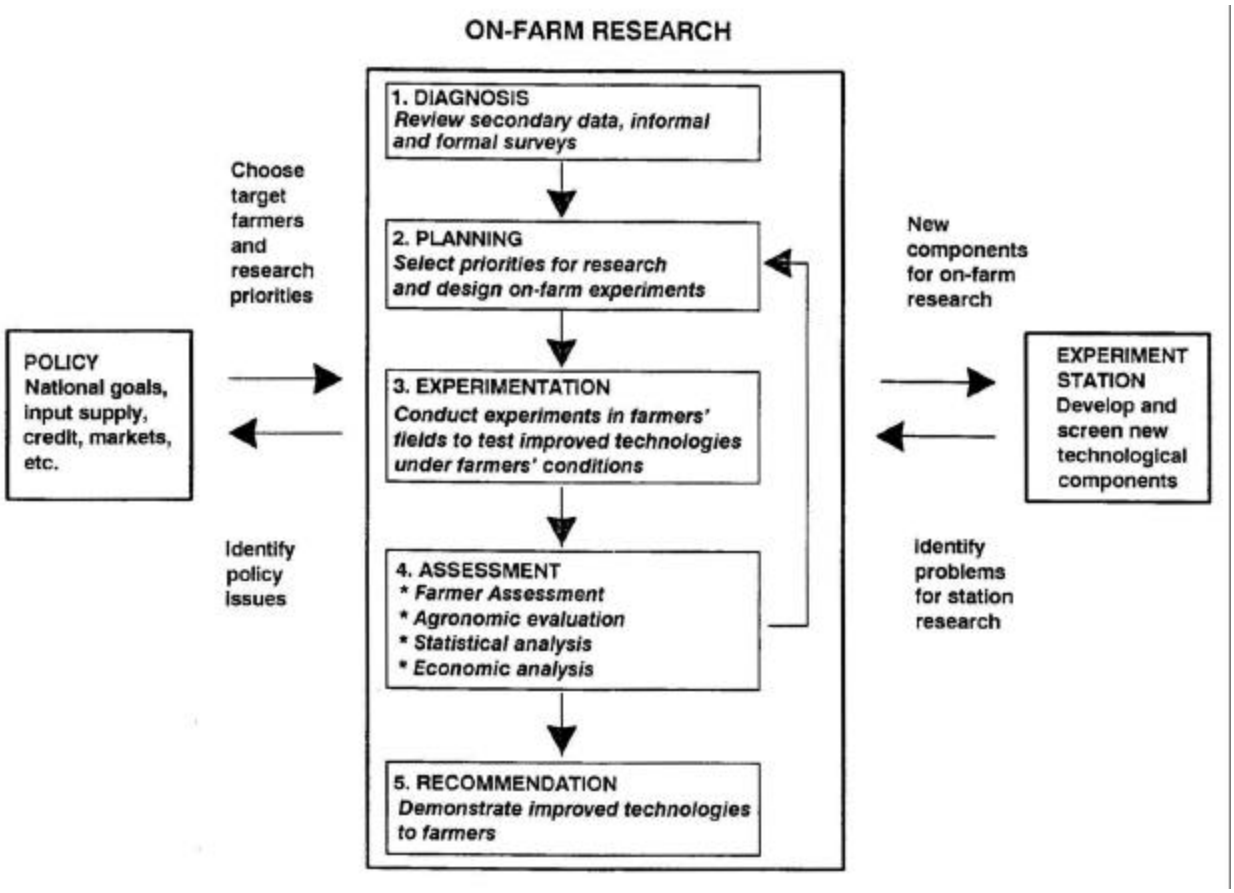
Programs promoting BNF technology should not mislead farmers into thinking that inoculation can benefit their crop system in any way except by providing more nitrogen. All inoculation trials and tests, whether they are on the farm or at the experiment station, are really testing whether nitrogen is limiting the productivity of the legume crop. This principle must be considered when examining legumes growing in farmers' fields or planning experimental programs to determine the need to inoculate legumes.

THE PROCESS OF DEVELOPING RECOMMENDATIONS TO FARMERS TO INOCULATE THEIR LEGUME CROPS

Technology can improve the productivity and welfare of the farm family. New technologies must be appropriate if they are to be adopted by farmers. An appropriate technology is socially and economically feasible in the context of the existing farm system, and there is a biological or physical need for the technology. The process necessary to make appropriate recommendations to farmers on the use of improved technology is long but not necessarily difficult. The planning process requires a realistic understanding of the technology, considering both the potential and limitations of the technology. An intimate knowledge of local conditions is also required.

Figure 7-1 is from a handbook titled, *From Agronomic Data to Farmer Recommendations: An Economics Training Manual*, Mexico D.F. (CIMMYT, Economics Program Mexico D.F.). The figure shows the process required to recommend a new technology to farmers. There are important elements of this process which must be addressed: 1) selecting an appropriate technology; 2) testing the technology under realistic farm conditions; 3) considering economic and social factors that may affect the acceptance of the technology by farmers. The following is a discussion of the important aspects of **Figure 7-1** in relation to inoculation of legumes.

Figure 7-1. Stages of on-farm research. A logical sequence for developing farmer recommendations to inoculate legumes and assess the benefit farmers derive from inoculation. The process required to recommend new technology to farmers. Reproduced from, *From Agronomic Data to farmer Recommendations: An Economics Training Manual*, with permission from CIMMYT, Economics Program.



Inoculation Technology and National Agricultural Goals

Research on the response to inoculation usually meets several existing national goals in agriculture. Reducing farm production costs, reducing environmental hazards, reducing importation of agricultural inputs, or import substitution of certain food commodities, are all national goals for agriculture. Inoculation technology has the potential to meet many of these national goals.

An Example. When high yielding varieties of rice and wheat were introduced, the use of nitrogen fertilizer was encouraged through a government subsidy of the fertilizer price. Although the programs were successful, the national governments have decided that the subsidies must be reduced and ultimately eliminated.

Farmers have come to rely on nitrogen fertilizers, even for legume production, since the price of fertilizer has been so inexpensive. Many national agricultural policies now call for the extension service to find alternative production methods that are less reliant on nitrogen fertilizers.

Experiment Station Results: Useful Preliminary Tests of Inoculation Technology

Experiments at research stations can produce valuable preliminary results on whether legumes may respond to inoculation. With the greater experimental control at the station, it is possible to detect smaller yield effects of inoculation than in farmers' fields, and examine other inputs that may affect the inoculation response.

Experiment station yields are usually greater than in farmers' fields, and therefore the response to inoculation may be greater than the farmer can get in his own field. It is important that recommendations are not based only on the results of a few trials at experiment stations. There is a vital need to validate the technology on the farm. In some cases, there may be an even greater response to inoculation on the farm, since rhizobia may have already been introduced to the experiment station in the past. With experiment station trials, extension workers gain valuable experience handling inoculants, growing the legume crop, and designing inoculation trials. These experiences will all improve the quality of later on-farm trials.

An Example: A representative from an inoculant producer approaches you to try a new inoculant for groundnut. You use the inoculant in two formal field experiments. The experiments are well managed. The yields in these trials are approximately 50% greater than local farmer yields, and there is a statistically significant response to inoculation. Your experience indicates that except for better management of the experiments, the conditions on experiment stations are similar to many farms in the region. These positive results mean that there is a possibility of obtaining a response to inoculation in the field, and further research planning is justified. Still, these results do not mean that the farmer will benefit from inoculating groundnut.

Diagnosis: Survey of Existing Data and Preliminary On

Farm Observations of Legumes in the Field.

Deciding to plan on farm trials. At this stage the extension agent needs to make a preliminary survey to determine whether inoculation technology may be appropriate to selected farmers. The survey can be based on existing data from trials, and on a survey of the status of BNF in the farmers' fields. First, groups of farmers who may benefit from inoculation must be identified.

Recommendation domains: Identifying groups of farmers with similar conditions who will benefit from inoculation technology. **Recommendation domains** are groups of farmers that have similar crop systems, management, climate, and soil. We expect that farmers within a recommendation domain will benefit from inoculation in a similar way because of the common conditions on their farms. They can be identified through on-farm surveys.

An Example: Inoculation produced a yield increase in groundnut at the experiment station. The extension agent decides further investigation is warranted, and a survey of 40 farms where groundnut is planted after rice is planned. Interviews with farmers are conducted and groundnut crops are examined. An initial tabulation of the results follows:

Table 7-1. Nodulation and apparent nitrogen status of groundnut crops following rice in Abung Timur.

Leaf Color		Nodulation		Effective Nodules	
Green	Yellow	Yes	No*	Yes	No
		Number of Farms			
32	8	26	14	22	4

*indicates less than 10 nodules per plant.

Table 7-1 indicates that only eight of the 40 farms had apparent nitrogen deficiency in their groundnut crops. If the extension agent only looked at leaf color, he may conclude that nitrogen deficiency in the groundnut crops is not frequent, and therefore further investigation on the value of inoculation is not necessary.

When nodulation is considered, the conclusions are different. Nodulation occurred on only 26 farms, and effective nodulation was observed on only 22 of those farms. There is an inconsistency between the observations of nitrogen deficiency and nodulation status of the groundnut crop. (Review **Module 6** for the factors that affect the nitrogen and nodulation status of the crop.)

The on-farm interviews indicated that none of the farmers inoculated. Nodulation must be from native strains in the soil. The survey included a description of the crop history and management. The following is a summary of **additional data** from the 40 farms:

Table 7-2. Crop management effects on nodulation of groundnut following rice in

Abung Timur.

Crop Management		No. farms	Leaf Color: Green	Leaf Color: Yellow	Nodulation: Yes	Nodulation: No	Effective: Yes	Effective: No
Applied Fertilizer Nitrogen:								
	Yes	12	12	0	0	12	0	0
	No	28	20	8	25	3	20	5
Years Between Groundnut in Crop Cycle*								
	1-2	22	21	1	18	4	18	0
	>2	18	11	7	7	11	2	5

>2 years in crop cycle includes farmers planting groundnut for the first time.

When data about farm management is considered, the reason for nitrogen deficiency and lack of nodules is clearer. In this case, the extension agent generated two separate **Recommendation Domains**; application of fertilizer nitrogen and number of years between crops of groundnut. Farmers using fertilizer nitrogen do not have nodules on their groundnut, but their crops are healthy. There was a higher proportion of farms with nitrogen deficiency (yellow leaves), plants with no nodules, or ineffective nodulation, when groundnut was planted infrequently or for the first time.

From simple observations in the field and proper farmer interviews, the extension agent can define the farmer groups that are most likely to benefit from inoculation technology. The extension agent can now formulate experimental plans based on particular groups of farmers. Based on management, two groups of farmers become candidates for on farm inoculation trials: farmers applying nitrogen fertilizer who will benefit from lower production costs if BNF can substitute for N fertilizer; and farmers who plant legumes infrequently.

This type of survey is simple, and can provide an extension agent with valuable information on the status of BNF on the farm. While the information gathered at this stage is not quantitative, it forms a useful database to identify groups of farmers likely to benefit from inoculation.

Planning a Research and Demonstration Program on the Farm
During research planning, priorities are established to test inoculation technology at the farm level. Proper planning is important to ensure that experiments are appropriate within the context of existing farm operations. Groups of farmers (Recommendation Domains) with similar physical, biological and social environments are further defined at this phase.

Variables in addition to inoculation should be considered, including farm practices, and physical and economic conditions. From **Module 6**, we know that benefits from inoculation are increased when other management inputs are used by the farmer.

Example: If the groundnut crops of your farmers were green but very poorly nodulated, you might conclude that another factor in the environment was limiting yield of the crop. Soil test data may indicate that P was low in the soils. During the planning stage, these facts and observations should be considered. The farm trials might include P fertilization in addition to inoculation as treatments in the experimental design.

How many variables should be tested in on-farm trials? Designs should be simple, use methods that are easy for the farmer, and practical, so that many trials can be conducted. Farmers do not usually adopt many new practices at one time. It is important that the number of treatments to be tested be kept to a minimum.

How many trials are needed to test a technology? Many observations are required to overcome problems with random variation between farms. The variation interferes with measuring differences between treatments. It is difficult to develop a recommendation to a defined group of farmers with less than 15 trials. More trials are recommended, but the number required to make valid recommendations varies with:

1) the extent of the recommendation domain
2) the size of the response to inoculation in the recommendation domain
3) variability of crop growth at individual farms from year to year

Only a small yield increase from inoculation will justify the farmer's investment in inoculant. Large numbers of observations increase the likelihood a small positive benefit from inoculation will be detected. Climate differences between years may change the results, so the trials should be conducted for more than one year.

Remember that once the farmer has conducted a trial and used inoculant, his field will be in a new recommendation domain.

An Example: Based on the preliminary survey groundnut farmers were selected as a group likely to benefit from inoculation. Observations indicated BNF in groundnut crops was dependent on management. Two recommendation domains can be identified: farmers who currently apply nitrogen fertilizer to their groundnut, and farmers who do not apply nitrogen. These two groups were selected because the benefits from inoculation and the cost of production for the two systems are different, and they will require different treatments to develop a recommendation on whether farmers should inoculate. Information on crop history and management should be collected at the selected farms so yield results

from the on-farm trials can be interpreted properly.

Trials testing the response to inoculating groundnut:

I. Farmers not applying nitrogen fertilizer.
Question to be answered by the trial: Do farmers planting groundnut after rice benefit from inoculation with rhizobia under existing management practices?

Proposed design:

1) Treatments:
   a) Inoculated
   b) Uninoculated
2) Inoculation method: Two-step seed coating; 10% sugar solution sticker; 300 g inoculant per 65 kg seed.
3) Management: Standard farmer practices
4) Replications within farm: 3
5) Number of farms: 15
6) Response measurement:
   a) Seed yield
   b) Seed protein
   c) Nodulation
   d) Leaf color

II. Farmers using nitrogen fertilizer as a standard practice.

Question to be answered by the trial: Can inoculation with rhizobia increase yield, and substitute for application of nitrogen fertilizer to groundnut following rice?

Proposed design:

1) Treatments:
   a) Inoculated
   b) Uninoculated
   c) Uninoculated plus nitrogen fertilizer

Note that this is not a complete factorial experiment where every combination of treatments is used. The question to be answered by the trials is not whether inoculation **and** fertilizer nitrogen increase yield. The question is whether inoculation increases yield and can substitute for fertilizer nitrogen.

2) Inoculation method: Two-step seed coating; 10% sugar solution sticker; 300g inoculant per 65 kg seed.
3) Management: Standard farmer practices
4) Replications within farm: 3
5) Number of farms: 15

6) Response measurement:

   a) Seed yield
   b) Seed protein
   c) Nodulation
   d) Leaf color

Information to collect from on farm interview:

1) Inoculation history
2) Management: planting density, fertilizers, cultivar, field preparation, date of planting and harvest.
3) Crop history: five year history of species and management
4) Soil type: observations of local classification, measure pH
5) Farmer's knowledge of inoculation

Conducting Inoculation Trials on the Farm: Some Basic Principles

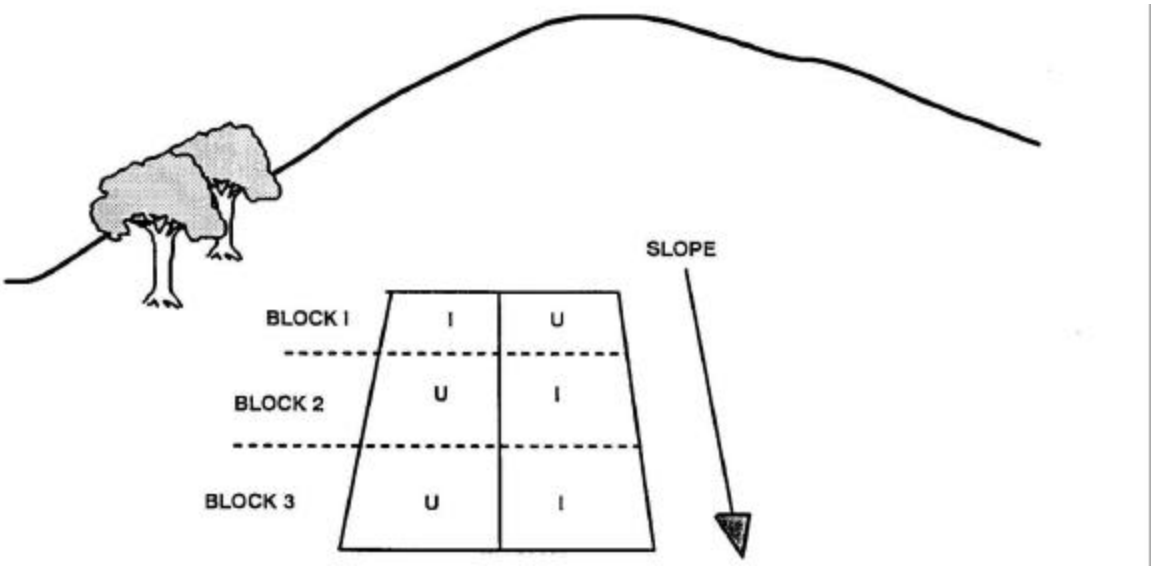
Exact instructions for conducting on-farm inoculation trials cannot be provided since local environmental and social conditions affect the design and execution of the trials. Local experience with other on-farm trials should be considered. The following are some suggestions and factors to consider when conducting on-farm inoculation trials:

1) In many cases inoculation technology is so new that the extension agent will have to work closely with each farmer to ensure that the inoculant is handled and applied properly. The extension agent should consider holding training sessions on inoculant storage, application and monitoring the effects of the inoculant on crop growth.
2) When conducting inoculation trials the extension agent must know that the inoculant used is of good quality. The inoculation trials should not be used to test inoculant quality. Laboratory methods to test inoculant quality are more effective and much less expensive than field trials.
3) Application rates and methods of inoculation should consider farmer cultural practices for the legume. For example, if the farmer usually uses fungicides on his groundnut seeds, he should apply them in the inoculation trial. The extension agent should then recommend soil inoculation instead of seed inoculation, unless he knows that the fungicide will not affect the rhizobia on the seed.
4) The inoculation trial should use rates of inoculant that the farmer can afford, but still meet minimum quantities required for good nodulation. It is better to ensure delivery of good quality inoculant to farmers than to recommend excessive rates of inoculation.

5) Care should be taken during planting inoculation trials. It is easy to contaminate the uninoculated treatment with rhizobia from the inoculant. Seed for the uninoculated plots should be kept away from the inoculation process. It is helpful if the uninoculated plots can be planted and covered before the inoculation and planting of seed for the inoculated treatments; however, planting uninoculated treatments first is only acceptable if the entire trial can be planted within a few hours.

6) Use repeated observations (replications) of treatments on a single farm, and take the average or mean values of these replications. The soil in farmers' fields is often variable. Using the mean of several observations gives a more accurate indication of the response to inoculation at a particular site. The repeated treatments should be put in **"blocks."** For example, in **Figure 7-2**, the paired Inoculated and Uninoculated treatments are placed in Blocks along a slope. The higher portion of the slope may have poorer soil due to erosion. By arranging the experiment along the slope, the conditions within each block are similar, even if conditions between blocks may be different. Putting replications in blocks reduces error in the statistical analysis of on-farm trials.

Figure 7-2. Treatments are repeated and placed in blocks on a slope.



Assessment of Inoculation Trial Data

Assessment of inoculation trials takes place on three levels:

1) farmer acceptance
2) statistical evaluation
3) economic evaluation

All three levels of assessment must be approached rationally within the context of the existing farm system.

Assessment by the farmer. The extension agent researcher needs to include the farmer in the assessment of the experimental results and consider the farmer's observations on both the practicality and benefits of using inoculant. Farmers' doubts about the use of inoculant must be addressed. Many times these doubts can be overcome through informational campaigns, but farmers' observations may lead to the need for additional research.

An example: Groundnut farmers apply fungicide to the seed. The inoculation treatment in the trial called for a liquid application of inoculant to the soil rather than seed coating. After the harvest, the farmers say that the inoculation treatment increased yields in many cases, but there is agreement that application of liquid inoculant to the soil is too much work. Further research is then needed to identify application methods or compatible fungicides that will make inoculation acceptable to farmers. In this case the on-farm trials return to the planning stage.

Statistical analysis of on-farm inoculation trials. Statistical analysis helps the researcher evaluate reliability of the data collected. Based on that reliability, the researcher can then apply economic principles to determine the financial benefit farmers can expect from inoculation. Statistical analysis is a useful tool. Through probability statements, the reliability of the treatment differences are determined. Statistical methods compare the difference in yield between inoculated and uninoculated crops to the amount of random or unexplained differences in the trials.

Non-Parametric statistics are a relevant method to evaluate the response to legume inoculation in series of on-farm trials.

What are Non-Parametric Statistics? Non-Parametric Statistics are simpler to use than Analysis of Variance (ANOVA) techniques, commonly used to analyze single and multi-site farm trials. Based on our experience, ANOVA techniques usually require a yield increase from inoculation of about 200 kg/ha to be considered statistically significant. Non-parametric statistics detect significant responses to inoculation in series of farm trials, based on the frequency responses observed, rather than the magnitude of the yield increase. Sometimes the yield increases due to inoculation may not be considered statistically significant by ANOVA techniques, but the non-parametric statistics do not require that the data meet assumptions of normality required by the ANOVA.

Most Non-Parametric statistical methods are based on a system where data are ranked according to their magnitude, and then assigned a number indicating their rank. Non-Parametric statistics are often called Ranking Tests. Tables are then used to determine whether the differences between inoculated and uninoculated crops are statistically significant. You would not use this method to analyze the data from a single farm trial, but rather for analyzing combined results of a series of farm trials.

Wilcoxon's Signed Rank Test for Paired Data: There are many non-parametric statistical tests. The Wilcoxon Signed Rank Test is particularly useful for inoculation trials, since the treatments of on-farm trials are always in pairs.

Table 7-3. Results of 15 on-farm inoculation trials of groundnut following a rice crop in Abung Timur. Data are the mean of three replicates.

Farm	Yield		Difference	Signed Rank
	Inoculated	Uninoculated		
	kg seed/ha			
1	961	909	52	10
2	980	930	50	9
3	1065	1090	25	-5
4	583	575	8	1
5	705	741	36	-8
6	1274	1038	236	15
7	872	840	332	6.5
8	626	635	9	-2
9	743	712	131	14
10	1294	1186	108	11
11	1052	1069	17	-4
12	798	766	32	6.5
13	1019	904	115	12
14	1489	1364	125	13
15	872	883	11	-3
Average	962	909	53	
Median response to inoculation 36 kg seed/ha.				

The median means 50% of the farmers had a response equal to or greater than 36 kg seed/ha.

An Example: Table 7-3 show data from 15 on farm trials using the same design presented earlier in this Module; two treatments (inoculated, uninoculated) and three replications of each treatment arranged in blocks on each farm. The differences between the average inoculated and uninoculated yields on each farm are ranked according to the instructions that follow. Analysis of variance of the individual trials indicate that the yield increases due to inoculation is significant only for Farm 6.

Even though the trials were conducted on farms from the same region and crop management and crop system was similar, the inoculation response and the yield of groundnut varies between farms. Average response to inoculation was only 53 kg seed/ ha.

The **median** means half the farms observed yield increases from inoculation greater than 36 kg seed/ha. Negative responses to inoculation occurred on five farms but averaged only 19 kg seed/ha. It appears that there should be a recommendation for farmers to inoculate, but the data should be statistically analyzed for the extension agent to be confident in his recommendation.

We recommend that the data be analyzed by simple non-parametric statistics. This evaluation will tell the extension agent how much confidence to have in using the results of these trials as a basis to recommend inoculation. The following is a simplified procedure for conducting a Wilcoxon Signed Rank Test.

Procedure to Evaluate Data from Abung Timur by the Wilcoxon Signed Rank Test:

1. The data must be **paired**. Only two treatments are compared.
2. Subtract the yield of Uninoculated from the yield of Inoculated. The difference is calculated without a negative or positive sign at this time.
3. Rank the differences according to their size. The lowest difference is given a rank of 1 (Farm 4) and the largest difference is given a rank of 15 (Farm 6).
4. When two differences are equal, assign each the average of the next two ranks. Farm 12 and Farm 7 both had a response to inoculation of 32 kg/ha. These two farms are each assigned the average of ranks 6 and 7 which is 6.5.
5. Assign negative signs to the ranks of farms where the yield of the uninoculated was greater than the inoculated (Farms 3,5,8,11,15) and positive signs to farms where there was a response inoculation (Farms 1,2,4,6,7,8,9,10,12,13,14).
6. Add the total of signed ranks for farms with positive and negative ranks. Sum of positive ranks = 98; Sum of negative ranks = 22.

If the sum of the negative ranks is ever greater than the sum of the positive ranks, there is no significant yield increase due to inoculation.

Is there a significant yield increase due to inoculation of groundnut in Abung Timur? Use **Table 7-4** and find the number of observation pairs (number of farms with inoculated and uninoculated yields). In this case the number of paired observations is 15. The "sum of ranks" in **Table 7-4** refer to the sum of the **negative** ranks.

If your sum of the **negative** ranks is **less than or equal to** the figures listed in **Table 7-4**, then you know that the increased yield due to inoculation is significant at the 95% or 99% confidence level. With 15 pairs of observations, **Table 7-4** indicates that your sum of negative ranks must be 25 or less to have 95% confidence level of probability that inoculation increases yield. Since the rank of the negative responses to inoculation in Abung Timur was 22, we can accept that "Inoculation increased yield of groundnut following a rice crop in Abung Timur" at a 95% level of confidence. In other words, we are 95%

certain that there was a real positive response to inoculation. The increase, however, is not significant at the 99% confidence level because the negative sum of ranks is greater than 16.

Table 7-4. Sum of Ranks for the Wilcoxon Signed Rank Test at the 95% and 99% Levels of Confidence. When the sum of negative ranks (negative response to inoculation) is equal or smaller than numbers in the table, inoculation had a significant positive effect on yield.

	Confidence Level	
Number of Observations	95%	99%
Pairs	- - - - - - - - - - Sum of Ranks - - - - - - - - - -	
7	2	0
8	2	0
9	6	2
10	8	3
11	11	5
12	14	7
13	17	10
14	21	13
15	25	16
16	30	19

Source: Snedecor, G. and W. Cochran. 1967. Statistical Methods, 6th edition. Iowa State University Press. Ames Iowa, USA.

What if there are more than 16 pairs of observations?

1. A table is not required for the Wilcoxon Signed Rank Test with more than 16 pairs.
2. If the sum of negative ranks is greater than the sum of the positive ranks, there is no significant yield increase due to inoculation.
3. When the sum of negative ranks is less than the positive calculate the following:

i: $u = n(n+1)/4$; where n = number of trials

ii: $s = \sqrt{(2n + 1)u/6}$

iii: $Z = (u - R - 0.5)/s$; where R = sum of negative ranks

4. If Z > 1.64 then inoculation increased yield at the 95% confidence level.

What level of confidence is required for on-farm inoculation trials? There are no rules dictating the level of confidence that should be obtained before accepting that yield increases are significant. The level of confidence in any experimental program should reflect the risk to the farmer if the analyses produced incorrect results. If new technologies being tested require large investments, then the extension agent should require more statistical confidence in the on-farm trial data. When the risk to the farmers is low, as in the case of recommending inoculant technology, the extension agent does not need a high confidence level to recommend the technology.

What does the non-parametric analysis tell us about the yield of the inoculated and uninoculated crops?

Non-parametric statistics are not used to estimate the average response to inoculation. Non-parametric statistics indicate the confidence that the **median** response to inoculation is greater than zero. The data of **Table 7-5** has a median response to inoculation of 36 kg seed/ha. This means half of the farmers had a response to inoculation of 36 kg/ha or more. In this case, the median value is less than the average increase of 53 kg/ha. The median more accurately predicts the yield increase a farmer can expect if he inoculates.

The Economic Benefit from Inoculation

Farmers invest in new technology only if they are convinced there is a positive economic return to the investment. There is no guarantee that any input the farmer uses will increase his income above the cost of the input with each crop. For example, many farmers apply nitrogen fertilizer to their maize or rice crops. The economic benefit they obtain from the nitrogen fertilizer may be negative in drought conditions where the crop cannot use the nitrogen applied. Investing in agricultural inputs involves risk to the farmer. It is the job of extension agents to develop management recommendations that will increase the farmer's income but minimize the economic risk the farmer must assume in adopting new technology.

A training manual on the economic interpretation of on farm trials titled "From Agronomic Data to Farmer Recommendations" is available from The International Maize and Wheat Improvement Center (CIMMYT). The following is adapted from this publication.

Costs, Benefits, and Risk of Inoculation

The relationship between cost, benefit, and risk determine the economic return for farmers using inoculant. Inoculation technology has some characteristics that are different than other agricultural technologies when evaluating this relationship.

Cost. Inoculant is inexpensive, and rarely exceeds 10% of production costs of the legume crop. Unlike other inputs, the cost of transporting the inoculant to the farm is very small. Inoculant does not require special application equipment, and requires very little extra labor to apply.

Benefits and Risk. As can be seen in **Table 7-3** and in **Module 6**, the **potential** benefits from inoculation are very large. In some cases, especially when there is a long history of cultivating the legume crop, farmers may not get yield increases when they inoculate. Therefore, there is a risk of not obtaining a response to inoculation. Negative responses to inoculation are usually small and due to random variation in field trials. The risk of inoculation failure is therefore limited to the cost of the inoculant. In contrast, with other inputs, the risk of failure sometimes means decreased yield in addition to the high cost of the input.

There is some debate whether economic analysis of inoculation technology is necessary because of the low cost of using the inoculant, minimum **adverse** risk, and the large potential returns that can be obtained. Many view the investment in inoculant as an inexpensive insurance for maximal BNF. Still, economic analysis is important if farmers are to be convinced to use inoculant.

Analysis of the Economic Benefit from Inoculation.

Marginal analysis is the calculation of **increased** income, above the cost of inoculation, due to investment in the inoculant. **The marginal analysis does not calculate the farmer's total income**. It only considers the **additional** money the farmer will make if he uses inoculant. In the following analysis, prices are in $US. Results will differ according to local prices of inoculant, labor, and grain.

An Example:

Costs of inoculation technology:

	Cost
1. Cost of inoculant/ha.	$2.75
2. Labor to inoculate ($0.50/h)	0.25
3. Materials (sticker, bags)	0.10
Total Costs/ha.	$3.10

Table 7-5. Marginal analysis of inoculation trials in Abung Timur, listing yield increase due to inoculation, price of groundnut, additional income due to inoculation (income increase), cost of inoculation, net income due to inoculation (income increase minus cost), and marginal rate of return (percent return on investment).

Median Yield Increase from Inoculation	Price of Groundnut	Income Increase	Cost of Inoculation	Net Income	Marginal Rate of Return
kg/ha	----------		$US	----------	%
36	0.25/kg	9.00	3.10	5.90	190

Marginal rate of return from inoculation is calculated by dividing net income by the cost of inoculant. This calculation, $5.90/$3.10 = 1.90, means that for each dollar invested in inoculation technology, the farmer can expect to get $1.90 **net** profit from inoculation, or 190% return on investment. Farmers usually require greater than a 50% rate of return to adopt a new technology, depending on economic conditions on the farm.

The marginal benefit from inoculation to these farmers is small. The marginal benefit is based on the median response to inoculation. At least 50% of the farmers will get a marginal rate of return on investment greater than 190%.

Break-Even Analysis. The **break-even** yield response is the level where increased income due to inoculation equals the cost of inoculant. To assess the risk that farmers assume by investing in inoculant, the proportion of farmers losing money (increases less than break-even yield) must be determined. The break-even yield response to inoculation is calculated by dividing the cost of inoculation ($3.10), by the price the farmer gets for each kg of seed.

Table 7-6. Break even analysis of farmers using inoculant on groundnut following a rice crop.

Cost of Inoculant	Price of Groundnut	Break Even Yield
---------- $US ----------		kg/ha
$3.10	$0.25	12.4
% Farmers above break-even yield = 60% (see **Table 7-5**)		

Nine farmers (60%) of the total from **Table 7-3** had increased net benefit from inoculation. This analysis of the proportion of farmers above the break even yield gives an idea of the risk that farmers face by purchasing inoculant.

The farmer is willing to take more risk if the potential gain is large or if the potential risk is small. The average increased income for nine farmers was $11.58. The average net loss of 6 farmers was -$5.65. The risk of loss then is 0.4 X $5.65 = $2.26 (the proportion of farmers losing money on inoculant X the expected loss). This risk of loss is extremely low compared to the potential gain the farmer can realize with inoculation.

The conditions on farms where there is a negative return on investment in inoculant should be studied. Perhaps these farmers belong to a different recommendation domain than the others. This information will help to design other trials that may improve the inoculation response on these farms.

The Farmer Recommendation.

From the preliminary survey, field trial data, and economic analysis, there are strong reasons to recommend inoculation to farmers growing groundnut after rice in Abung Timur. This recommendation can be made with confidence, since the majority of farmers will benefit from inoculation. The recommendation can be based solely on yield and current costs and prices. Other benefits not considered in this analysis include greater protein content of seed and greater N fertility of the soil with inoculation.

DIAGNOSES OF BNF PROBLEMS AND MEASURING THE RESPONSE TO LEGUME INOCULATION

Information on how environment and management influences the response to legume inoculation is necessary in the design of on-farm and experiment station trials. It is important to remember the **Law of the Minimum**, the appearance of **effective** and **ineffective** nodules, and how **mineral nitrogen** and **native rhizobia** in the soil affect the response to inoculation, when you evaluate BNF in the field or design research programs to test inoculation response.

Following is a discussion of three levels of diagnosing problems with legume BNF and measuring the response to legume inoculation. These levels are 1) observations in

farmers' fields; 2) greenhouse pot tests; and 3) field experiments. There are more details in the Demonstrations for **Module 7**. The uses of the various techniques are discussed in the following sections.

Examining Legumes in the Field: Simple Diagnostic Methods to Assess the Status of BNF in the Farmer's Field.

Preliminary surveys of farmers' fields are important to detect problems with BNF. These observations are useful to help the extension agent develop an experimental plan and identify a `recommendation domain' that requires further research.

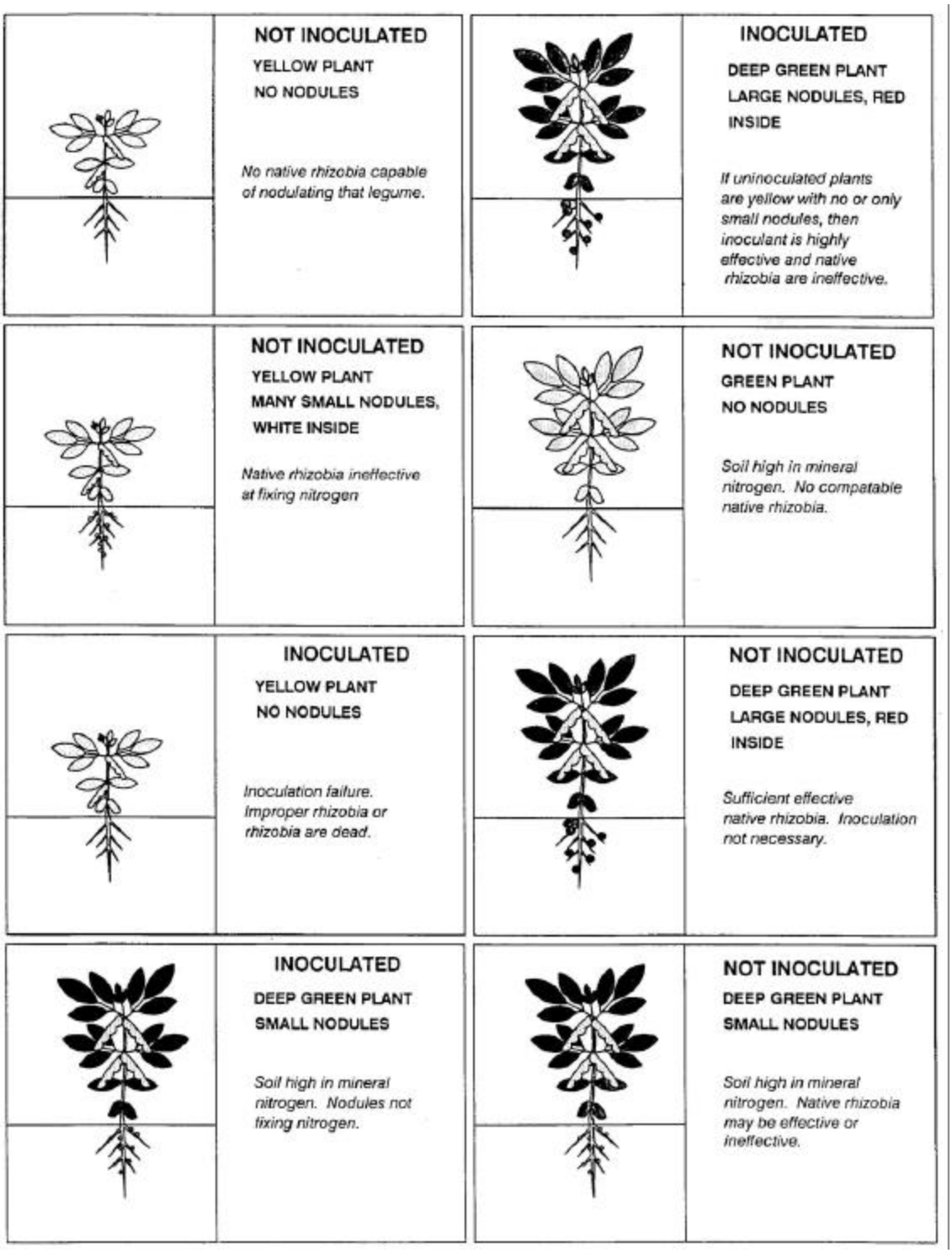
Figure 7-3 provides a useful summary of situations that extension agents may see in the field. The descriptions are divided into two management categories: 1) inoculated; 2) uninoculated. Information on whether or not the farmer inoculated is needed to interpret observations. Although the descriptions of the field situations are simple, it is often possible to make recommendations to the farmers. For example, nodulation failure and nitrogen deficient plants almost always indicate that there are no rhizobia in the soil or in the inoculant. Information on management, soil, and climate factors will also help the extension agent to interpret his observations. By comparing observations on crops on farms in the same area, you can detect whether differences in management may affect BNF.

Elements of a preliminary survey of farm fields:

1. Crop history
2. Inoculation history
3. Management
4. Soil and climate information

Conducting the on farm interview. Most farmers want to help extension agents obtain the information they require. In fact, many farmers are so eager to please extension agents that they sometimes give answers they think the extension agent wants to hear. It is very important to ask questions that need more than a 'yes' or 'no' answer.
An example: During the interviews on farms in Abung Timur the survey asked the frequency of groundnut cultivation in the last five years. The extension agent should not ask farmers: "Did you plant groundnut last year?" The farmer may think you look favorably on groundnut cultivation and try to provide you with a favorable response. Ask the farmer: "What crops have you planted following the rice crop during the last five years?" This approach is more likely to produce accurate information for your survey.

Figure 7-3. Situations commonly observed in farmers fields and their explanations.



Examining Legume Crops in the Field

Standardizing Observations. Observations should be standardized for a meaningful survey. Criteria used to make an assessment must not vary between farms.

It is simple to standardize leaf color observations. This observation is important to detect nitrogen deficiency in crops. Always examine leaves at the same position on the plant. Usually, it is best to examine the most recently developed leaf. Compare the leaf color with color samples that have been selected as standards. Try to compare farms that have crops at the same stage of maturity, since the nitrogen status of a crop changes with the stage of growth.

Observations on the nodulation of legumes growing on the farm provides important information about the rhizobia in the soil. Random plants in the field should be sampled. The nodules on most species will become detached if plants are pulled from the soil. Instead, the root system must be lifted from the soil with a digging tool, and the soil gently removed. Develop a rating system for the nodulation of the crop, including effectiveness, size, distribution, and abundance. It is important to have experience with the nodulation characteristics of the species you are working with before you design the rating system.

Variation in the Field. Variation of soil N, crop growth, and native rhizobia within the farmer's field is common and must be considered. Sample plants at random throughout the field. Knowledge of previous management practices on the farm will help to select sampling areas. If the farmer inoculated part of his farm, the introduced rhizobia will not necessarily move to other parts of the farm.

Greenhouse Methods to Assess the Response to Inoculation.

Greenhouse tests of the response to legume inoculation are simple diagnostic tools. They can provide the extension agent with information on how inoculation is likely to affect the yield of legumes in the field. Demonstration 2 for **Module 7** describes how to conduct inoculation trials using soil from farmers' fields.

Results from properly performed pot experiments agree well with results from field experiments, and they can be confirmed in the field. The advantage that pot experiments have is that the extension agent can easily test inoculation response in many different soils.

An Example: Observations of groundnut growing in fields after rice cultivation in Abung Timur indicated nodulation varied greatly between fields. Information from the on-farm interview suggested that management practices such as nitrogen application and frequency of groundnut cultivation affected nodulation of the crop. Two basic questions about the need to inoculate groundnut can be easily answered with pot experiments. 1) Will farmers who currently apply nitrogen to their groundnut crops benefit from inoculation when no nitrogen is added?; 2) Does the frequency of groundnut cultivation affect the response to inoculation by groundnut?

A suggested design: Select farms for soil sampling based on management: 1) farms applying fertilizer nitrogen to groundnut and cultivating groundnut every year; 2) farmers planting groundnut every year without adding fertilizer nitrogen; 3) farms with no groundnut cultivation in the last four years.

At least three farms in each management category should be selected. Collect enough soil from each site to fill six pots and handle according to instructions in **Module 7** Demonstration 2.

There are at least two treatments required for this experiment: inoculated; uninoculated. There should be at least three replications for each treatment, and more if possible. Dry weight or total nitrogen yield should be determined after harvesting the plants. The same non-parametric statistical analysis can be performed on the pot tests as the on-farm trials.

The extension agent should also consider using greenhouse pot experiments to test the performance of inoculant under different levels of management. Since the work involved with pot tests is much less than in the field, the extension agent can often obtain preliminary results that indicate further research needs to increase legume yields.

Other Survey Techniques that Indicate the Need for Farmers to Inoculate Legumes.

Recent advances in technology have developed survey techniques that predict the response to inoculation. These techniques are more quantitative than the on-farm survey presented earlier. The techniques require that researchers count rhizobia in the soil. While the counting technique is not difficult, it does require special training and facilities that are not available to most extension agents.

The techniques to predict the response to inoculation are very cost effective compared to field trials. If there is a need for such a survey in your region, you should contact professionals at the national university with training in BNF research. They can get assistance to conduct a survey from NifTAL.

THE FORMAL FIELD EXPERIMENT TO TEST INOCULATION RESPONSE.

There is information on how to conduct a formal field experiment in **Module 7** Demonstration 1. This type of experiment is usually large, with numerous treatments. It has a well defined experimental design for both controls and statistical analysis. This type of trial is more suited for experiment stations than on-farm sites.

Experiment stations trials are good opportunities to demonstrate the potentials of the latest technologies. Farmers can also learn about the interaction between management variables, since these experiments can have a more complex design than the on-farm trials.

An Example: Some farmers of Abung Timur plant groundnut after rice on highly weathered soils. These soils are red in color and yields are usually low. The soil science department of the local University says that these soils are deficient in phosphorus. There are currently no recommendations on the management of these soils for groundnut cultivation. The objectives of the experiment are:

1. Test the response to inoculation by groundnut in these soils.

2. Develop data that describes the response of groundnut to various rates of P fertilization.

3. Test the interaction between P fertilization management and the response to inoculation.

Experimental Design: Split plot design: Main plots (four P fertilization levels including control); Sub-plots (inoculated; uninoculated); 4 replications.

Data collection: Soil P test values; P and N concentration in leaf tissue at flowering; biomass at flowering, mid-pod fill, maturity; nodule dry weight at flowering.

This design is presented in more detail in **Module 7** Demonstration 1.

Review, Discussion and Case Studies

1. Form a strategy to make inoculation recommendations to farmers in your district:

   What are the crop systems?

   Which are the recommendation domains most likely to benefit from inoculation?

   Design an evaluation and systematic research program including data analysis.

2. What are some of the social and economic considerations when promoting inoculation technology in your area?

3. Compare the use and promotion of inoculation technology with other agricultural technologies in your district. Evaluate several agricultural technologies in terms of potential return and risk to the farmer, and compare to the use of inoculation technology.

4. The extension service has recommended that starter nitrogen be applied to legumes at planting. Develop a research program to answer:

   1) Is this recommendation valid?
   2) Can inoculation substitute for starter N?
   3) What are the costs and benefits of each technology?

SUGGESTED LESSON PLAN FOR MODULE 7

TIME: 2-3 hours +

OBJECTIVES:

Knowing how to measure the response to inoculation and how to evaluate SUccess or failure of BNF in the field. Knowing the process required to make recommendations to farmers to inoculate their legumes.

MATERIALS:

Demonstrations 7/1 and 7/2

Training Aids for Module 7

STEPS:

1. Display key concepts and other appropriate-training aids.

2. Much of the practical material in this module can be combined with the field experience gained in Module 6.

3. Lectures should be frequently interspersed with discussion and question and answer sessions. Situational case studies from the participant's actual experience win provide the kinds of information necessary to arrive at good recommendations for farmer's decision making.

KEY CONCEPTS

Training extension workers in applied BNF technology can help farmers make appropriate decisions about inoculating legume crops.

There is a logical process that leads to appropriate farmer recommendations to Inoculate:

1. identifying problems with BNF In the field

2. designing appropriate tests to validate the value of Inoculation

3. economic interpretation

4. training and extension work

5. recommendation to farmers to inoculate

Recommendation domains are groups of farmers who are likely to benefit from inoculation technology in a similar way. Farmers belong to a recommendation domain when conditions on their farms are similar.

Inoculation is an inexpensive technology; the risk of monetary loss to the farmers is low and the potential gain is very high.

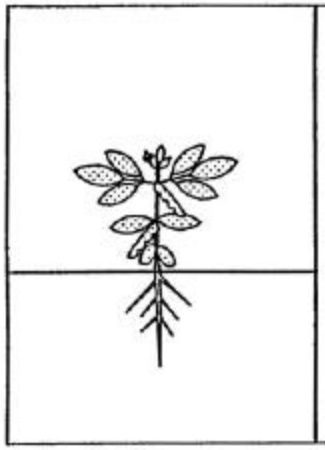
MODULE 7

Analysis of on-farm trials to test the response to inoculation requires special but simple approaches.

There are many ways to test the crop response to Inoculation, Including experiment station field experiments, greenhouse pot tests, soil surveys, and on-farm trials. Each has specific advantages.

Non parametric statistics are an appropriate method to evaluate the response to Inoculation In on-farm trials.

Economic analysis of Inoculation technology compares the cost of inoculation to the increased revenue the farmer gets from Inoculation.

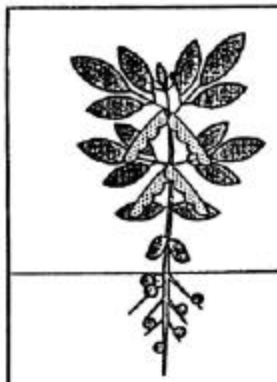


INOCULATED

YELLOW PLANT

NO NODULES

Inoculation failure. Improper rhizobia or rhizobia are dead.

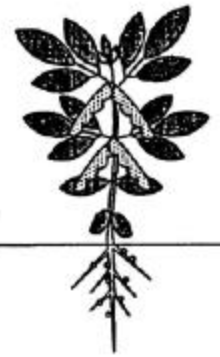


NOT INOCULATED

DEEP GREEN PLANT

LARGE NODULES, RED INSIDE

Sufficient effective native rhizobia. Inoculation not necessary.



INOCULATED

DEEP GREEN PLANT

SMALL NODULES

Soil high in mineral nitrogen. Nodules not fixing nitrogen.



NOT INOCULATED

DEEP GREEN PLANT

SMALL NODULES

Soil high in mineral nitrogen. Native rhizobia may be effective or ineffective.



NOT INOCULATED

YELLOW PLANT

NO NODULES

No native rhizobia capable of nodulating that legume.



INOCULATED

DEEP GREEN PLANT

LARGE NODULES, RED INSIDE

If uninoculated plants are yellow with no or only small nodules, then inoculant is highly effective and native rhizobia are ineffective.



NOT INOCULATED

YELLOW PLANT

MANY SMALL NODULES, WHITE INSIDE

Native rhizobia ineffective at fixing nitrogen

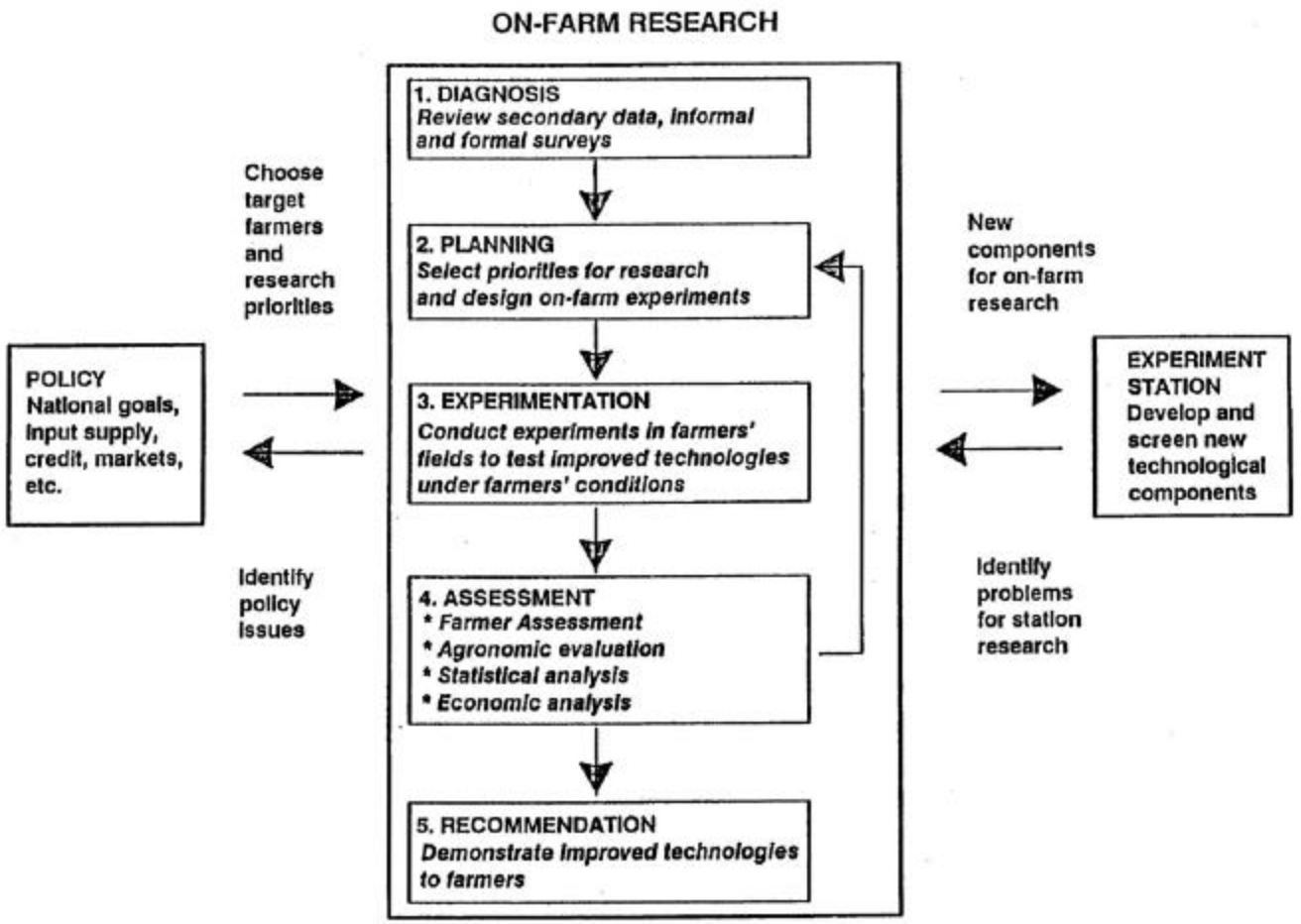


NOT INOCULATED

GREEN PLANT

NO NODULES

Soil high in mineral nitrogen. No compatable native rhizobia.


ON-FARM RESEARCH
1. DIAGNOSIS
Review secondary data, informal and formal surveys
2. PLANNING
Select priorities for research and design on-farm experiments
3. EXPERIMENTATION
Conduct experiments in farmers' fields to test improved technologies under farmers' conditions
4. ASSESSMENT
* Farmer Assessment
* Agronomic evaluation
* Statistical analysis
* Economic analysis
5. RECOMMENDATION
Demonstrate improved technologies to farmers
Choose target farmers and research priorities
Identify policy issues
POLICY
National goals, input supply, credit, markets, etc.
New components for on-farm research
Identify problems for station research
EXPERIMENT STATION
Develop and screen new technological components

MODULE NUMBER 8

COMMUNICATION SKILLS & TECHNOLOGY TRANSFER

SUMMARY

The purpose of this module is to enhance communication skills through knowledge and practice. Communication is the key to transferring BNF technology. Only through the effective effort of extension specialists and agents can farmers gain the skills necessary to use this technology.

First, this module will be a refresher course on the importance of your role as a BNF trainer and the need to know some techniques to communicate for better understanding. Second, we will look at blocks to communication and do some exercises which point out problem areas. Third, we will practice some skills. Fourth, participants will work together with course instructors to plan a strategy for the future efforts in BNF technology transfer. Finally, participants will present a mini-course in BNF and Inoculant Technology.

KEY CONCEPTS

- YOU are the key to successful BNF technology transfer
- Information (New Knowledge) is flexible, alive, and easy to transport
- People want to learn something that will improve their lives
- Apply new information to past experience and real life — make it meaningful
- People don't hear or understand everything that is said
- People learn differently
- Repetition is good
- Using a variety of teaching methods is most effective
- Planning is a key to success

IMPROVING COMMUNICATION SKILLS

YOU are the key to successful BNF technology transfer.

Transferring BNF technology successfully depends on the capability of persons like you in the extension service to train agents and farmers, to diagnose BNF problems in the field, formulate feasible solutions, and to spread the knowledge of BNF. (Review Demonstration 8/1).

Participants in this course have been selected because they are already considered to be

in positions that will allow them to expand the knowledge of BNF by training others. You were probably selected because you have certain skills and attributes that make you capable of teaching others.

These attributes also have to do with your professional commitment to extension work. Ask yourself this series of questions, and make a mental note of whether you can answer yes or no to each question.

Can you help people to help themselves and enjoy doing it?
Do you believe farmers are intelligent and capable people?
Are you willing to learn from farmers?
Do you enjoy the success of others?
Do you resent criticism of farm people?
Do you believe there is always a quicker, easier, cheaper, safer, or better way to do a job? Are you anxious to look for it and get it in use?
Are you a creative thinker?
Are you able to discipline yourself?
Is your goal in life service rather than wealth?
Would you rather be a king maker than a king?
Are you sympathetic to farmers and their difficulties and willing to listen to their problems, even when you would rather be doing something else?
Do you feel a sense of responsibility to the people you serve, over and above office hours and your pay packet?

Source: Adapted from Handbook for Extension Work, Flores, Bueno, Lapastora, SEARCA, The Philippines, 1983.

The yes answers to these questions imply an attitude that fits with extension work. It probably also means you are a person able to use the training in this course to effectively transfer BNF technology to others.

Knowledge grows when we transfer technology

Life's experiences, including growing up on a farm or receiving an education in agriculture or extension work, prepares us to communicate with others. As you communicate with extension agents or farmers in the transfer of BNF technology, you will become an important person in assessing the successful use of BNF by farmers. With this information, you will have an even larger body of knowledge that can have a wider dissemination.

Imagine the power that is gained by the BNF message when one successful farmer tells another or when you are able to use that example in your presentations.

Information is a renewable resource; it is flexible, easy to transport, and alive. It grows and becomes refined with use. Once a farmer experiences success in the field, the knowledge is available to him forever. It can be taught to children and other farmers and moved from one farm to the next. People are anxious to improve their lives. That brings us to the next concept.

People want to learn something that will improve their lives

If farmers understand the importance of using BNF to improve their lives, they will be happy to learn. We know farmers have specific goals.* Understanding farmer's goals is useful when we want to transfer BNF technology. Extension workers must consider these goals and accept the constraints in achieving those goals the farmer desires.

1. Farmers are primarily concerned with assuring an adequate food supply for their families. They may produce most of the food their family consumes or market a portion of their output and use the cash to purchase food. Farm enterprises also provide other necessities for the farm family, either directly or through cash earnings. In addition, the farm family is a member of a community and has obligations to that community. To meet these requirements, farmers often manage a very complex system of enterprises that may include various crops, animals, and on-farm work. Although this manual concentrates on improving farmer's lives through transferring BNF technology, it is essential that legume inoculation be compatible with the larger farming system.

2. Whether farmers market little or most of their produce, they are interested in the economic return. Farmers will consider the costs of changing from one practice to another and the economic benefits resulting from that change. (You can apply in this context the discussion of the benefits of using BNF in **Module 6** and economic benefits in **Module 7**.) Farmers will recognize the benefit of harvesting more seed when they inoculate legumes. They also realize they must give up some time, effort, and cash to buy inoculant. Farmers will compare the yield benefits gained to the things lost in the form of labor and cash given up. What farmers are doing in this case is assessing the difference in net benefits between practices—the value of the benefits gained minus the value of the things given up.

The farmer's most important consideration will be the risks of trying something new versus the benefits. The farmer must be convinced there is little or no risk in inoculating legume seeds. The discussion of causes for inoculation failure covered in **Module 6** are important issues for consideration by extension agents and farmers as they assess risk. Farmers attempt to protect themselves from risks of loss in benefits and often avoid choices that subject them to risks, even though such choices may yield higher benefits than less risky choices do. Recall the discussion of risk assessment from inoculation technology in **Module 7**. The farmers preference for stable returns rather than the highest possible returns is easy to understand.

*Adapted from Agronomic Data to Farmer Recommendations, CIMMYT, 1989.

Activity (Demonstration 8/1): Describe the typical extension agent you will be working with. Describe the typical farmer you expect will be a potential user of BNF technology.

Apply new information to past experience and real life—make it meaningful

Information is most readily received if the extension agent can link it to something that farmers use, enjoy, desire, or dislike. For example, inoculation makes the most sense to a farmer who grows legumes for food or market. The desire for a better life is a strong motivation toward learning something that will give them more food on the table and a potential cash crop. This same farmer dislikes not being able to provide for his family. Thus, link the transfer of BNF technology as close to the farmer's life experiences as possible.

Think about the extension agent and farmer described earlier. How does the description of extension agents and farmers help to link BNF technology to their experience?

Activity: Make lists answering this question.

BLOCKS TO COMMUNICATION

People do not learn everything that is taught

We learn only 20% of what we hear. Only limited information can be held in the mind at one time. One estimate is that people can only think about six things at one time. When we hear something that requires time for thought we miss other things that are said. Further, we all can acknowledge we must work to focus our mind on a lecture, especially when the conditions are uncomfortable or we have been sitting for a long time.

Seeing doubles the amount of information we gain. Providing ways for learners to see is one of the more enjoyable parts of communication for many of us. Drawing graphs, sketches, and lists; showing slides or videos; or assembling displays is a good method of adding the visual element to teaching. It is also useful to provide written materials because reading can reinforce knowledge through seeing.

Activity Demonstration 8/2): The Secret. Participants pass a short secret orally it should be written out by the facilitator) through the line of all the people present. Let the last person write what he or she heard, and then compare the two secrets.

We can use 40% of what we hear and see.

We can use 80% of what we see, hear and experience. Again, the addition of another

element doubles the amount we can gain in information received. Doing is critical if a technique like inoculation is to be learned. Doing must be a part of any BNF training course. Theoretical information is good to know, and diagnostic skills are useful to farmers, but the ability to inoculate seeds is essential.

People don't understand everything that is said

Often when we try to communicate to teach, the information is quite abstract. What do we expect from the learner? The following exercise will give us some understanding of what a learner may experience.

> Activity (Demonstration 8/2): Have participants form pairs. Hand out cards with abstract figures drawn on them. Have one person describe to the other how to draw the hidden figure. Any instructions are okay as long as they are verbal—no hand signals. Have them compare the two figures and discuss how their instructions might have been more effective. Give pairs a chance to report.

Aside from the obvious difficulties of transferring information as we just experienced, there are other problems. The use of a special language or jargon connected to a particular subject often inhibits information transfer. BNF uses a special language. Many terms we use are unusual and words used in one context might have a different meaning in another. The problem is that these are usually the best words to transfer meaning. How do we overcome this? Each teacher must use judgement in deciding how best to communicate. Three examples of methods for overcoming this problem are: 1) introduce the new vocabulary as you go along (best with extension agents who need to understand written background materials); 2) select different words that you know will convey the same or a similar meaning; or 3) simplify the material to the extent that the use of jargon will not be necessary. You are the best judge of how and when to use these or other techniques to overcome the problems of effectively teaching the difficult concepts of BNF.

Teaching involves more than just giving someone information. Each audience has unique characteristics in the way they learn, which makes it important for extension workers to understand the special characteristics of adult audiences.

Teaching Adults Requires Special Considerations

The three most important factors in teaching adults are:

Respect. To communicate well with adults, respect is essential. Children can be taught in a very directive way, however adults must feel honored and respected as a person, not for what he or she knows but for themselves. Respect, then, is a way of communicating to the sense of feeling. Everyone can think of a situation in which they felt disrespected. It is obvious that whether the learners are extension agents or farmers, ensuring that they feel respected will be of great benefit when transferring the BNF message.

Immediacy. Learners must see how they can use their new knowledge, skills and attitudes immediately. They need to carry away from a learning experience a tangible gain. In our case this could be the knowledge of how to inoculate seeds, how to obtain inoculants, how to tell if a legume has nodules, or even a packet of inoculant and instruction sheet.

Experience. As discussed earlier, providing a learning experience where participants can relate the new knowledge to something known and valued is essential. Adults learn best when their learning is directly related to their own life experience.

Adults who are ready to learn are the easiest to teach. It is important to be sensitive, however, to physical realities. Speak clearly and slowly and use visual aids as much as possible because vision declines steadily after age 14, with a marked decline in the middle age (45-55) and hearing ability declines steadily from 14 years on. Men lose ability to hear higher tones and women lose on the lower tones as they grow older.
One last thought on creating the right learning environment. It is up to the teacher to make the learning environment a safe place to practice skills and experiment with new knowledge and ideas. Feeling safe gives people a chance to experiment and make suggestions which may even challenge the teacher. Nothing is more rewarding to a good teacher than seeing learning grow before their eyes. If an appropriately respectful environment is provided, people will automatically feel safe and ready to learn.

Repetition is good

Often when we are teaching others, we think they will become bored if we repeat information. There is another way to look at this belief. Consider that we remember only a portion of what we hear, see and do. How then do we create situations in which people will learn? We can repeat the same information in several ways.

Perhaps you have realized that our approach in this course has been to present the same information differently as each module was taught. We used repetition. If you have learned well, it may have been because we used that technique. Our model for teaching has been to **SHOW IT**; **TELL IT**; **DO IT**.

For example, in the last module you saw slides as part of the lecture, you were reminded of things from previous modules, and you actually diagnosed BNF problems. You saw, you heard, and you did. We used repetition of the same material to reinforce your knowledge. When teachers have little time to complete a training session, they often forget to take time to use repetition as a teaching technique.

It is also very important to evaluate whether learning has taken place. This is best done by questioning, but always keeping in mind the conditions of successfully teaching adults. What are some other ways of gaining attention from learners?

Use a variety of teaching methods

No two people are alike and no two people learn alike. They need to be approached using

a variety of teaching techniques. The following table shows that the use of more teaching methods increases the rate of technology transfer.

Table 8-1. Increasing teaching methods enhances technology adoption.

Number of different teaching methods used	Percentage of people who adopted the practice.
1 to 2	45%
3 to 4	64%
5 to 7	95%

Source: *Handbook for Extension Work* , Flores, Bueno, Lapastora, SEARCA, Philippines, 1983.

By using the five senses of sight, sound, taste, smell, and touch, it is easier to influence people to accept new ideas such as legume inoculation. Extension teaching methods are classified as written (bulletins, leaflets, news articles, etc.); spoken (meetings, home visits, office or telephone calls, radio); objective or visual (result demonstrations, exhibits, posters, charts, slides, video tapes, etc); and spoken and objective or visual (method demonstration or informational meetings).

The teacher must adjust his or her style and teaching role according to the objective they are attempting to achieve, i.e., transfer information primarily by lectures or new techniques by demonstration. However, there are other useful teaching roles. **Table 8-2** lists all the roles and gives some information on each.

Table 8-2. Teaching roles

Role	Activity	Goal/Comments
Lecturer	Presents oral material	Don't forget that lectures are usually not enough
Coordinator	Organizes efforts of more than one person or group	Extension specialists spend much of their time coordinating the efforts of groups
Demonstrator	Shows how to do something	Essential in communicating BNF technology
Guide	Asks questions Responds to questions	Helps people to learn for themselves. Good to use with adult learners
Facilitator	Gives instructions and guides learning as needed	Learners have both safety and freedom in this setting
Observer	Gives instructions and then simply stands back and lets learner teach him or herself	Allows us as teachers to step back and see where the learner is and how much he or she can do on their own

This gives us an opportunity to become familiar with using the training aids at the end of each module. We have provided you with materials and suggestions on their use. You are very creative people and will come up with innovations in their use that we haven't thought of. The next activity (DO IT) will make use of these training aids.

> Activity: practice using flip charts and training aids at the end of modules. Be clear about your teaching role, facilitator, lecturer, etc.

PLANNING

Planning is a key to success

First, we can use a relatively simple set of seven steps* that have helped trainers and managers prepare presentations and training events, conferences, and workshops. It has been proven a useful instrument in planning any meeting or workshop.

1. HOW: steps, activities, materials (Modules 1-7)
2. WHO: participants, trainers, resource persons
3. WHY: the situation (BNF technology transfer)
4. WHEN: the time frame (1 to ? days)

5. WHAT FOR: objectives (get farmers to use legume inoculants, extension agents to teach farmers, etc.)
6. WHERE: site
7. WHAT: content

*Adapted from learning to teach: training of trainers for community development, Save the Children, 1989

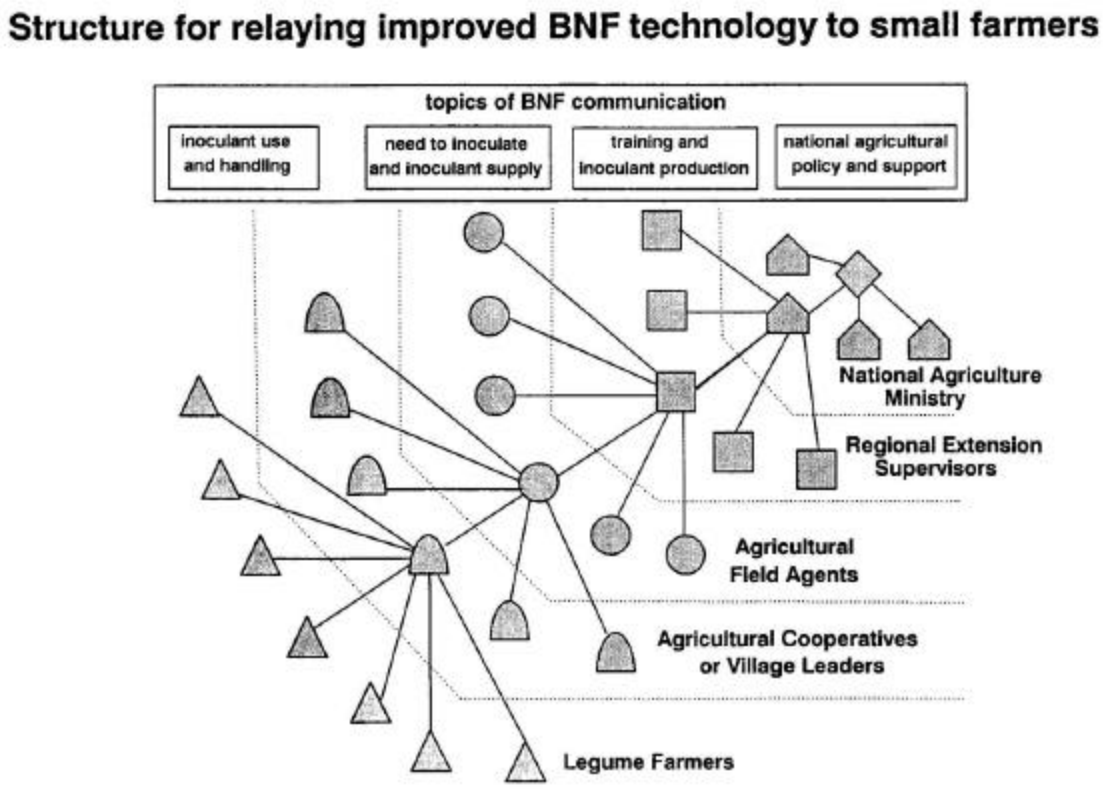


Figure 8-1. The structure of BNF communication for extension of technology.

In order to be successful in teaching others, we must plan carefully. One aspect of planning that is imperative for BNF technology transfer is deciding how to start the training process. Perhaps you have thought about this and of some of the problems that will have to be overcome before you begin. Each situation is different and requires a slightly different approach. We can, however, expect to consider the following in most cases:

Your agency and the national commitment to the importance of BNF technology
Your own resources of time, program flexibility, and funding
Your agency's resources of time, program flexibility, and making funds available to you
The availability of materials, i.e., handouts and inoculant
Scheduling training sessions or field days to coincide with planting seasons or other events to ensure the greatest impact
Will a media promotion help to inform farmers that BNF is important and useful?

Developing a strategy is essential for transferring BNF technology. Each participant will be challenged to plan this strategy. People together have more power than people alone. Take advantage of your network of participants in this course as you return to your home agencies. Your combined strength can have a powerful impact for positive change and for getting the message of the value of adopting BNF technology to those who can use it.

Activity (Demonstration 8/3): Develop a strategy for BNF technology adoption.

Once the strategy for technology transfer has been planned, practicing teaching the technology is one way of reinforcing what has been learned. The last exercise for this module is the presentation of a mini-course. How much can actually be taught in seven 1/2-hour lessons?

Activity: Divide into groups of two or three. Each group selects a module to present. Use the lunch hour and early part of the afternoon to prepare. Present in 15 min. to half-hour segments.

SUGGESTED LESSON PLAN FOR MODULE 8

TIME: 2 hours +

This module can be enriched by adding exercises designed to help people become comfortable with each other.

OBJECTIVES:

Knowing that technology cannot be transferred without people who have the knowledge and willingness to teach what they have learned.

MATERIALS:

Training Aids for Module 8

Large sheets of paper

Note paper or copies of the exercises on page 8-2 and the abstract figure drawing in the training aids.

STEPS:

1. Display key concepts and other appropriate training aids.

2. You will decide how deeply to go into this material. Administering the self-test on page 8-2 is one of the most important exercises. A review of adult learning patterns will be a simple exercise that would also be useful.

3. The learning evaluation for this module is the process of planning strategies for technology transfer. If participants are excited about BNF technology and set as helping to solve the problems of farmers in their areas, you will have been highly successful in your teaching of these materials. CONGRATULATIONS!

KEY CONCEPTS

YOU are the key to successful BNF technology transfer.

Information (New Knowledge) is flexible, alive, and easy to transport.

People want to learn something that will improve their lives.

Apply new information to past experience and real life-make it meaningful.

People don't hear or understand everything that is said.

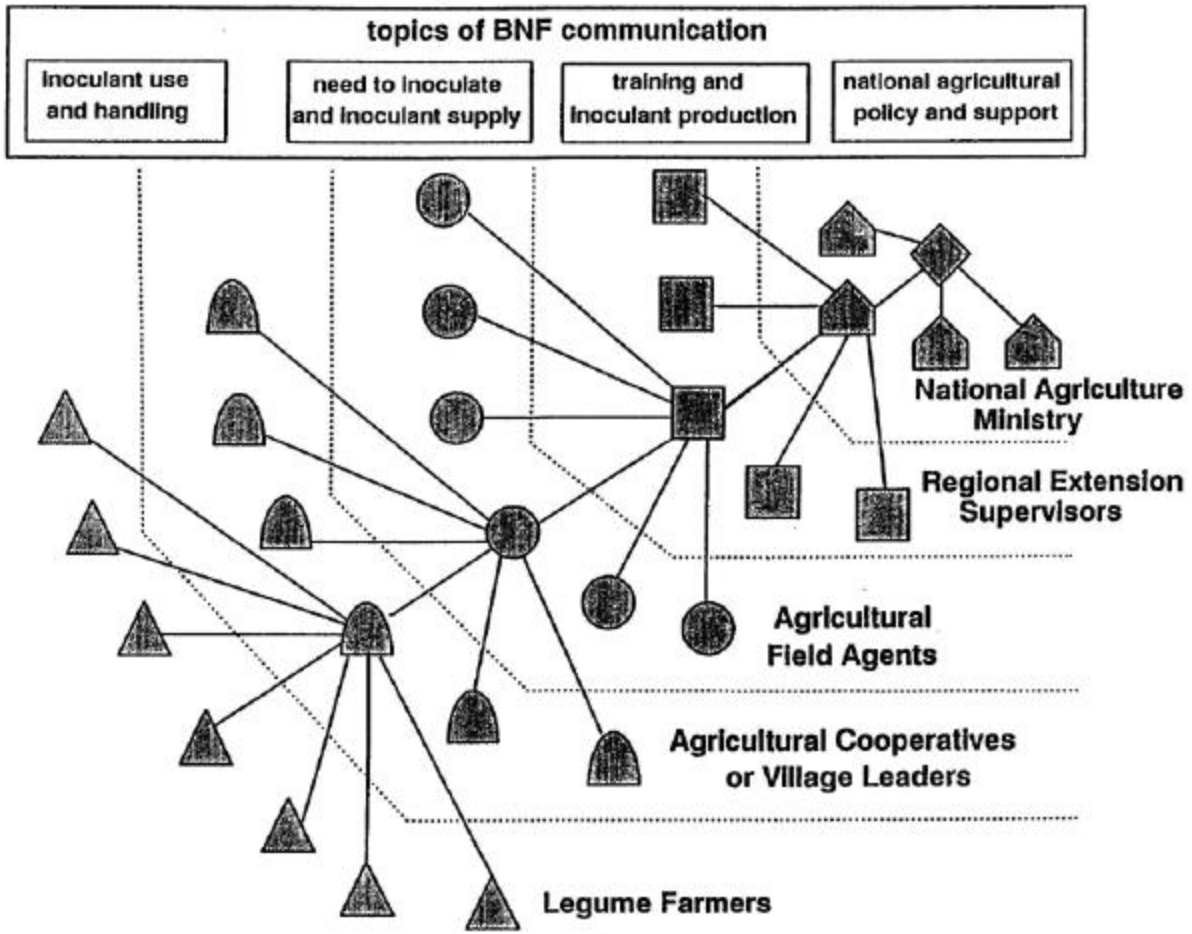
People learn differently.

Repetition is good.

Using a variety of teaching methods is good.

Planning is a key to success.

Structure for relaying improved BNF technology to small farmers



Can you help people to help themselves and enjoy doing it?

Do you believe farmers are intelligent and capable people?

Are you willing to learn from farmers?

Do you enjoy the success of others?

Do you resent criticism of farm people?

Do you believe there is always a quicker, easier, cheaper, safer, or better way to do a job? Are you anxious to look for it and get it in use?

Are you a creative thinker?

Are you able to discipline yourself?

Is your goal in life service rather than wealth?

Would you rather be a king maker than a king?

Are you sympathetic to farmers and their difficulties and willing to listen to their problems, even when you would rather be doing something else?

Do you feel a sense of responsibility to the people you serve, over and above office hours and your pay packet?

Use a variety of teaching methods

Table 8-1. Increasing teaching methods enhances technology adoption.

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From Handbook for Extension Work, Flores, Bueno, Lapastora, SEARCA, Philippines, 1983.

GLOSSARY

Anabaena azollae -This relationship is useful in rice-based crop systems throughout Asia.

Azolla-Anabaena **symbiosis** -A biological nitrogen fixation relationship between the aquatic fern Azolla and the cyanobacterium Anabaena azollae. This relationship is useful in rice-based crop systems throughout Asia.

Aeration -Supplying or charging liquid with a gas to be used in respiration.

Ammonia -A colorless gas produced in the manufacture of fertilizers and found in a wide variety of nitrogen containing organic and inorganic chemicals. In developing nodules, ammonia is needed for attachment to a compound provided by the host, forming an amino acid.

Ammonium (NH_4) -A chemical ion that is produced during BNF.

Bacteroids -Pleomorphic forms of rhizobial cells found in the nodules.

Biological Nitrogen Fixation (BNF) -The conversion by certain algae and soil bacteria of atmospheric nitrogen into organic nitrogenous compounds assimilable by plants.

Blocks -recommended division of test areas to ensure similarities in test conditions.

Break-even analysis -the level where increased income due to inoculation equals the cost of inoculant.

Caesalpinoideae -A subfamily of Leguminosae, with irregular flowers. One of the poorest nodulating subfamilies of Leguminosae.

Carpel -The central ovule-bearing female organ of a flower consisting of a modified leaf forming one or more sections of the pistil.

Competitive -Those strains of rhizobia that are faster at forming nodules than other strains.

Cover crops -A temporary crop, such as rye or clover,planted to protect the soil from erosion in winter and to provide humus or nitrogen when plowed under in the spring.

Cross inoculation group -A collection of legume species that will develop nodules when inoculated with the rhizobia obtained from the nodules from any member of that legume group.

Cycle -The completion of a series of events making a full circle.

Denitrification -When nitrate is changed back into nitrogen gas(N_2), permitting its return to the atmosphere. This is carried out by bacteria found in soil and water.

Dicotyledonous plants -One of the two major divisions of angiosperms, characterized by a pair of embryonic seed leaves that appear at germination.

Dusting method -The least effective method of seed inoculation and not recommended. Powdered inoculant is mixed with dry seed resulting in poor adhesion.

Effective -When the rhizobia and legumes are well matched and nodules form that will fix nitrogen.

Enzyme -Any of numerous proteins or conjugated proteins produced by living organisms and functioning as biochemical catalysts in living organisms.

Fertilizer use efficiency -The fraction of nitrogen applied that is actually taken up by the crop.

Flagella -Thread-like structures that make rhizobia motile.

Forage legumes -Legumes grown in pastures for animal feed.

Fungicides -Seeds are often coated with these chemicals for fungal control. Fungicides are usually harmful to rhizobia. Soil inoculation is recommended when they are used.

Grain -Cereal grasses or the small hard seeds or fruit from cereal grasses.

Green manures -A growing crop, especially a legume, that is plowed under the soil to improve fertility.

Grow out test -A method of testing the nodulation ability of an inoculant. Seeds of host legumes are inoculated and checked for nodulation after three to four weeks of growth.

Harvest index -The weight of grain or other economic yield divided by the weight of shoot and grain. Used to evaluate the benefit of legumes to the nitrogen fertility of soil.

Ineffective -When the rhizobia and legumes are not well matched and even though nodules may form, they will not fix nitrogen.

Infection process -The series of events whereby a rhizobia enters the root cells of a legume.

Infection tunnel (infection thread) -The passageway by which the bacteria moves through several root celllayers of the plant to the site where the nodule will develop.

Inoculant -The carrier material used to introduce rhizobia to leguminous seeds. The ratio of inoculum to carrier is 1:1 to 1:2, depending on the absorption ability of the carrier.

Inoculation -In Rhizobium technology, infecting soil or legume seeds with rhizobia.

Inoculum carrier -A highly absorbent non-toxic material used to mix with inoculum. Peat, finely ground or granular In texture, is the carrier most commonly used.

Inoculum -A broth culture of rhizobia used to make inoculant.

Inorganic N -Nitrogen derived from mineralization, e.g., N in the form of NO_3 and NH_4.

Insecticides and Herbicides -These chemicals are often applied in granular form to the furrow. They are only harmful to rhizobia when applied to the seeds directly.

Intercrops -The secondary crops growing between the rows of a principal crop.

Introduced rhizobia -The rhizobia put in the fields through farmer's inoculants.

Kwashiokor -Severe malnutrition occuring especially in children, characterized by anemia, edema, potbelly, depigmentation of the skin, and loss of hair or change in hair color.

Law of the Minimum -Yield in a farmer's field is limited by a single factor; only when that factor is added to the crop will yield increase.

Legume-rhizobia symbiosis -Intimate association of rhizobial bacteria and leguminous plants that leads to Biological Nitrogen Fixation (BNF).

Legumes -Any plant of the family Leguminosae, characteristically bearing pods that split into two valves with the seeds attached to the lower edge of one of the valves.

Limiting nutrients -The nutrient in the smallest supply determines the size of the farmer's yield. This nutrient is called the limiting nutrient since the amount of this nutrient determines the yield of the crop.

Marginal analysis -the calculation of increased income, above the cost of inoculation, due to investment in the inoculant.

Mimosoideae -A subfamily of Leguminosae with flowers collected into a dense head. The subfamily with the second highest incidence of nodulation.

Native rhizobia -Rhizobia that are already living in the soil.

Nitrogen mineralization -The conversion of soil organic N to inorganic forms of N.

Nitrogen gas (N_2) -The inert form of nitrogen found in the atmosphere which is converted to ammonium by BNF or by chemical fixation.

Nitrogen harvest index -A measure of the efficiency of recovery(harvest) of the total nitrogen in a crop.

Nitrogenase -An enzyme which enables rhizobia to convert N2 to NH3(ammonia).

Nodules -A small, knoblike outgrowth, such as those found on the roots of most leguminous plants.

Non-parametric statistics -Appropriate statistical analysis for a series of on-farm inoculation trials.

On-farm research -A logical sequence for developing farmer recommendations to inoculate legumes and assess the benefit farmers derive from inoculation.

Organic N -Nitrogen derived from dead and living organisms, e.g., N in the form of amino acids or proteins.

Papilionoideae -A sub family of Leguminosae with characteristic 'butter-fly' shaped flowers. The sub family with the highest incidence of nodulation.

Persistence -Referring to the survival of introduced rhizobia.

Photosynthesis -The process by which cells in green plants convert light to chemical energy and organic compounds from inorganic compounds, especially carbohydrates from carbon dioxide and water, and release oxygen at the same time.

Plant nutrient -The essential elements required by a plant for growth.

Plant infection tests -A method of estimating the number of rhizobia in inoculant or soil samples. A serial dilution is made of the sample and an aliquod of each dilution is added to a host plant. The resulting nodulation or absence of nodulation will indicate presence of rhizobia.

Promiscuous -A term used to describe the legume that can form symbiotic associations with rhizobia from many other hosts.

Range plants -Pasture legumes or other plants growing naturally in fields.

Recommendation domains -groups of farmers that have similar crop systems, management, climate, and soil. Farmers within a recommendation domain can expect to benefit similarly from inoculation.

Residual Nitrogen -The nitrogen that is left in the soil after a crop has been harvested and decomposition of soil organic matter has taken place. This residual nitrogen is then of benefit to the next crop.

Rhizobia culture -Growing rhizobia in a nutrient medium under artificial conditions.

Rhizosphere -The region around and close to the root.

Root hair -A thin hairlike outgrowth of a plant root, that absorbs water and minerals from the soil. It is on the root hair that rhizobia will enter the root.

Rotational crops -Changing crops from year to year to resupply the soil with nutrients that have been depleted.

Saprophytes -Organisms which live on the organic matter in the soil.

Seed Pelleting -Inoculated seeds are coated with a layer of powdered lime or phosphate. The pelleting material forms a hard coating around the inoculant as protection from adverse weather conditions, protection against soil additives, insects, soil acidity, etc.

Senescence -Aging and decaying, as in legume nodules.

Slurry inoculation -A seed inoculation method which requires a slurry made by mixing sticker with inoculant. This slurry is then coated on the seed.

Soil organic matter -Plant and animal residue that gradually decompose, releasing nutrients.

Starter Nitrogen -A small amount of nitrogen farmer's apply to their legume crop at planting.

Stover -The dried stalks and leaves of a cereal crop that remains after the grain has been harvested.

Strains -Rhizobia of the same species which are genetically distinct.

Swartzioideae -A small subfamily of Leguminosae that is relatively unimportant economically with nodulation not well known.

Two-step inoculation -A seed inoculation method in which seeds are first uniformly wetted with a sticker. Inoculant is then added and coated on the sticky seeds.

Vascular tissue -The connections that enable the host to feed sugars from photosynthesis to the rhizobia and the rhizobia to transfer fixed N_2 (ammonia) in the nodule to the plant.

Wilcoxon's Signed Rank test for paired data –A non-parametric statistical test useful in inoculation trials since inoculated and uninoculated treatments are paired on each farm.

Yeast mannitol agar –a solidified culture media of yeast sugar alcohol and mineral salts used in the culture of rhizobia in the laboratory

SLIDE NOTES AND EXPLANATIONS

1. M1/1 The Nitrogen Cycle. Gaseous nitrogen in the air is converted into a biologically useful form through biological nitrogen fixation in legumes and through chemical fixation in the fertilizer manufacture process.

2. M1/2 The Detailed Nitrogen Cycle. Crops require more nitrogen than any other plant nutrient. Nitrogen transformations in the biosphere are controlled by bacteria; nitrifying bacteria convert nitrogen in organic matter to ammonia and then nitrate; denitrifers convert nitrate in the soil back to atmospheric nitrogen; rhizobia convert this nitrogen back into ammonia within the root-nodule.

3. M1/3 The Leguminosae. The Leguminosae is the third largest plant family, with over 20,000 species represented in the temperate and tropical habitats, from herbs to large trees. Some of the worlds most important high protein foods are legumes, such as beans, soybeans, peas, peanuts and alfalfa.

4. M1/4 Rhizobia are Soil Bacteria. Rhizobia are rod-shaped soil bacteria which can be either free living or symbionts. When the proper legume root arrives in their soil habitat they can invade the root and eventually come to reside inside the host structure called a root-nodule. From inside the nodule, they carry out the process of biological nitrogen fixation.

5. M1/5 High Protein Products from the Symbiosis. The high nitrogen levels in a well nodulated legume give rise to high protein levels in the harvested plant. Soy sauce, tofu, and peanut butter are examples. Legumes such as peanut and soybean also produce high quality oils used in cooking.

6. M1/6 Matching the Plant and the Microbe. This field demonstrates two important points: 1) inoculation is necessary for proper growth of the plant (peanut, in this case) when the rhizobia are not present in the soil, and 2) different genetic varieties of the host require different rhizobia for an effective symbiosis.

7. M2/1 Subfamily Caesalpinoideae. A flower of *Bauhinia* sp. shows floral morphology typical of the species in the subfamily Caesalpinoideae.

8. M2/2 Subfamily Mimosoideae. Inflorescence of *Acacia farnesiana* which consists of small florets arranged to give a "head" common to species in the subfamily Mimosoideae.

9. M2/3 Subfamily Papilionoideae. "Butterfly" flowers of the subfamily Papilionoideae are typified by *Lathyrus* sp.

10. M3/1 What Rhizobia Look Like. These are rod-shaped bacteroides of *Bradyrhizobium japonicum* stained with fluorescent antibodies.

11. M3/2 Effective Symbiosis Nodule Color. Sections through effective nodules show the presence of leghemoglobin. Note the red color similar to human blood.

12. M4/1 The Infection Thread. Rhizobia enter the legume host usually through penetrating a root hair. The invagination of the host cell results in an "infection thread," by which the rhizobia travel to the site of the nodule primordia.

13. M4/2 Nodulated Soybean Root System. This soybean root system is covered with root-nodules. Within these structures are millions of rhizobia. It is within these nodules that nitrogen fixation occurs. The host expends a lot of energy maintaining these active nodules in return receiving ammonia which is converted to amino acids and proteins.

14. M4/3 Nodulated Peanut Root System. Nodule shape is determined by the host legume. Note the many smooth spherical nodules.

15. M4/4 Nodulated Birdsfoot Trefoil Root System.

16. M4/5 The Inoculated Seed. Farmers use the rhizobia by coating seed prior to planting with peat which carries the bacteria. Peat-based inoculants are available commercially to farmers in developed countries and increasingly in developing countries.

17. M4/6 Ineffective Native Rhizobia. The small plant on the left is a poorly nodulated alfalfa grown in a Washington state field. The native soil rhizobia were parasitic on alfalfa, and inoculation (in spite of severe competition) benefited the plants, as shown on the right.

18. M4/7 Inoculation Response in Soybean. This sandy soil in Florida showed a dramatic response to inoculation with soybean inoculant, as shown by

the rows on the left. The two rows on the right are uninoculated plants. The economic return in such a situation is quite high.

19. M4/8 Inoculation Response in Alfalfa. Perennial legumes such as alfalfa can be inoculated after planting. The middle and right plots were inoculated several months after planting, showing that perennial legumes can be rescued from nitrogen starvation. Annual legumes have too short of a growing season for this to work with them.

20. M4/9 Intercropping. The legume *Dolichos lablab* is used as an intercrop in this banana orchard in Honduras. It contributes nitrogen to the soil upon later incorporation and provides erosion control.

21. M5/1 The Slurry Inoculation Method. The following slides give an example of seed coating by the slurry method. First, measure corn syrup.

22. M5/2 Add peat based inoculant.

23. M5/3 Stir the mixture until a uniform slurry results.

24. M5/4 Measure seed into a roomy bucket.

25. M5/5 The slurry is added to the seeds which are stirred until seeds are well coated.

26. M5/6 Optionally, lime may be used for a protective coating after seed inoculation.

27. M5/7 A measured amount of lime is added to the seeds until they are uniformly coated.

28. M5/8 Seed Coating By the 2-Step Method. First, a measured amount of sticker material is added to seeds contained in a plastic bag. Then, the plastic bag is closed in such a way that as much air as possible is trapped in the bag. Vigorously shake the bag for one minute to uniformly wet the seeds with sticker. Next, the peat inoculant is added to the sticky seeds. Again close the bag and shake gently for another minute to coat the seeds with inoculant. Finally, the coated seeds are poured onto a clean surface, spread out and allowed to dry.

29. M6/7 Comparison Table. Soybean cultivation affects the number of soybean rhizobia in soil.

30. M6/7-2 Graphic: A Response Model. Factors controlling the response farmers can obtain by inoculating their legumes.

31. M6/7-3 Comparison of Nodule Amounts From Inoculated and Uninoculated Plants. Inoculation can increase the number of nodules on legumes. On the right, nodules from a system having poor nodulation on uninoculated legumes. Note the few, but relatively large nodules.

32. M6/7-2 Field Study View. Response to inoculation is evident by the size and color of plants.

33. M6/7-5 Starter N Benefit Chart. The benefits to starter nitrogen are a function of both the legume and the soil.

34. M8/1 You Are The Key. Introduce the concept of responsibility with this slide pointing out that each participant is responsible for their role in the BNF technology transfer process.

A series of self-explanatory text slides follows:

Communication & Teaching Skills:

35. M8/2 What Motivates People to Learn?

36. M8/3 What Adult Learners Expect

37. M8/4 How People Learn

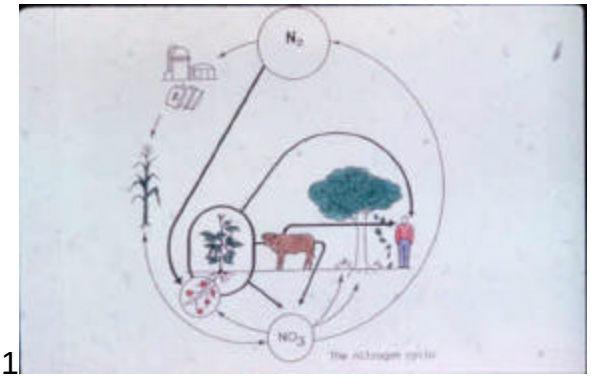
38. M8/5 People Remember

39. M8/6 Teaching Method

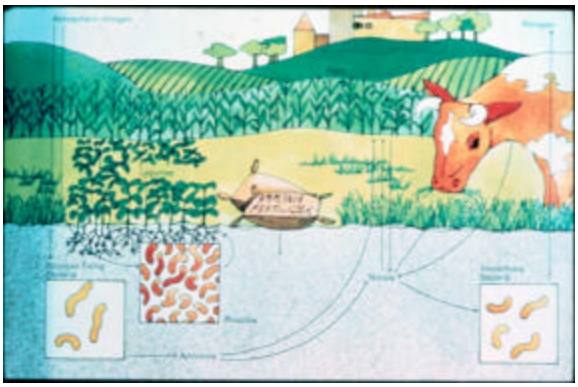
Planning Technology Transfer:

40. M817 When Planning, Systematically consider

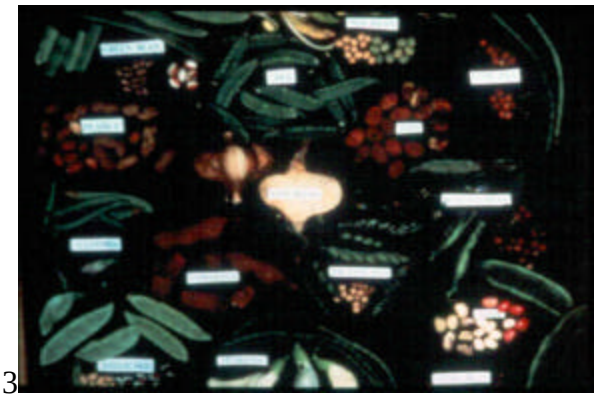
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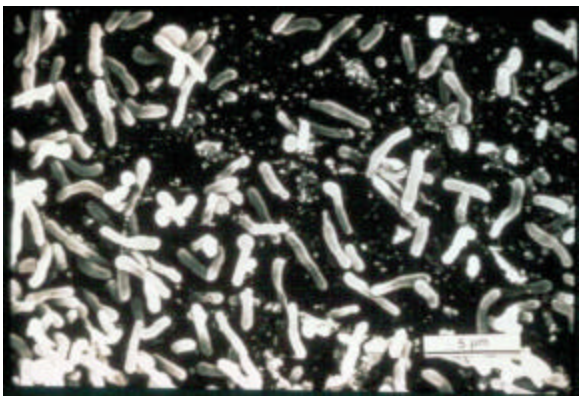
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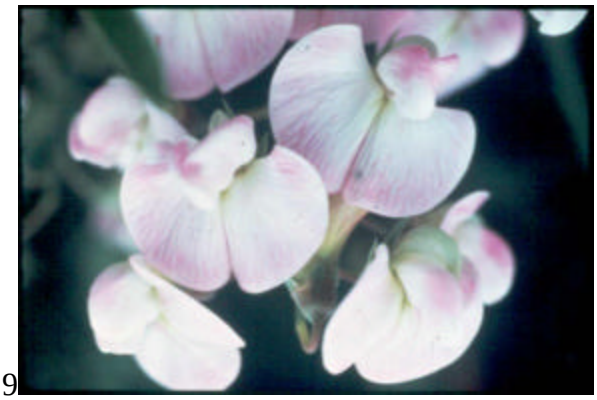


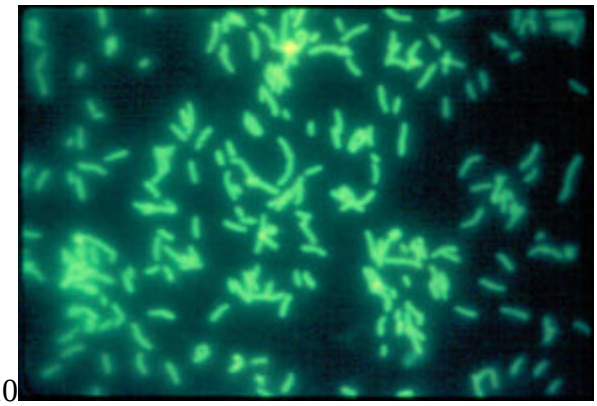
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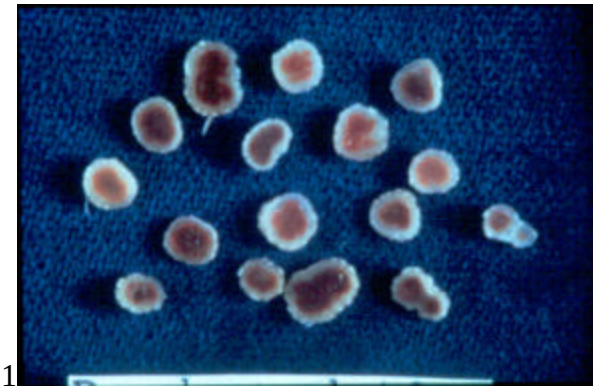


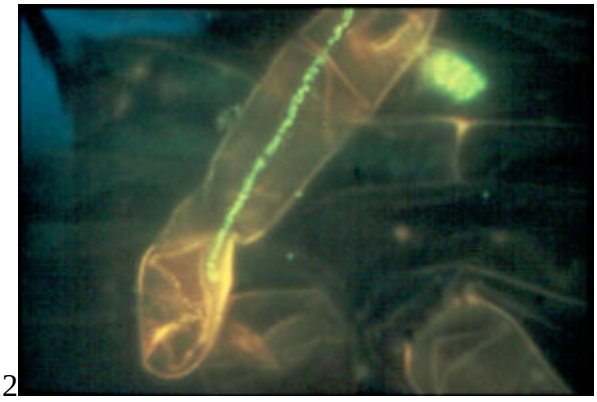
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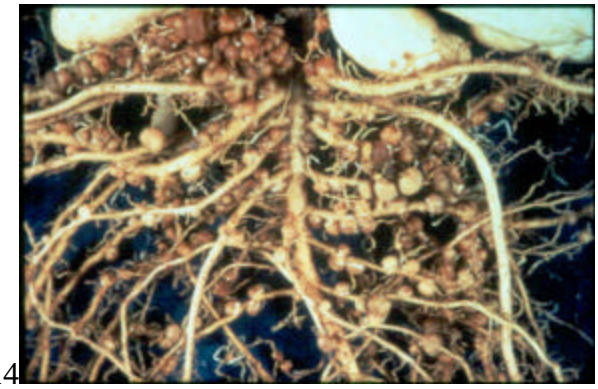
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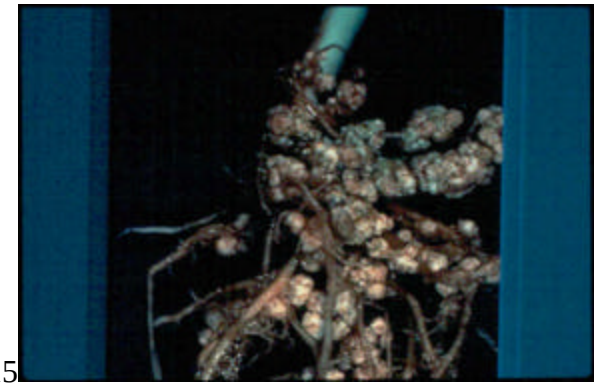
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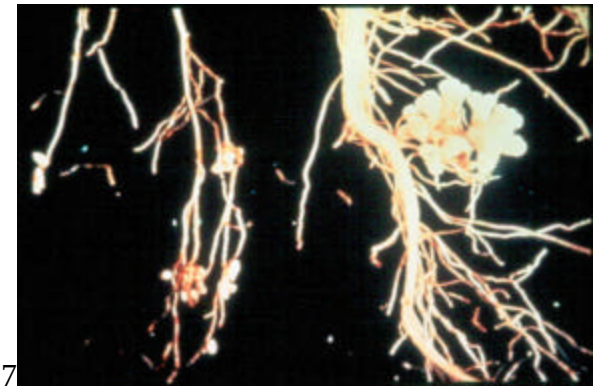
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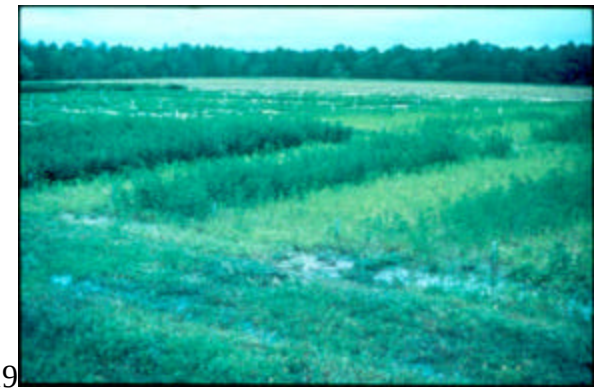
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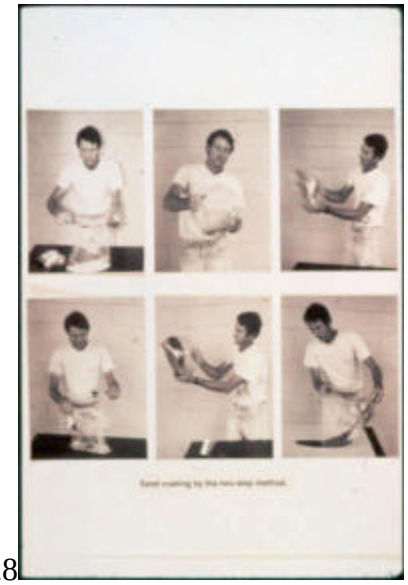
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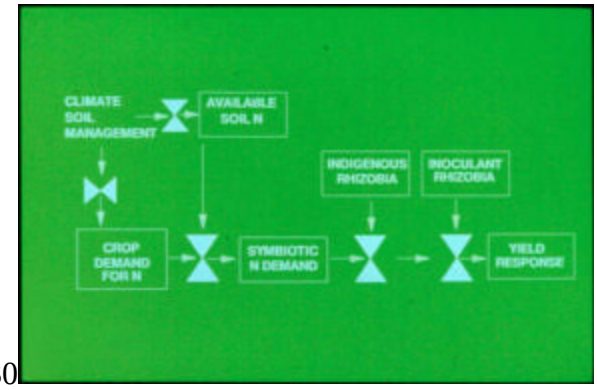
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Frequency Distributions of *Bradyrhizobium japonicum* populations in Sumatra in Relation to the Number of Years of Soybean Cultivation.

Number	Years 0	1	2-5	>5
g^{-1} soil	relatively frequency (%)			
0-10	61	60	0	0
10-100	21	40	33	27
100-1000	7	0	33	27
>1000	11	0	33	45

28, 5, 6, 11 observations for 0, 1, 2-5, >5 years.

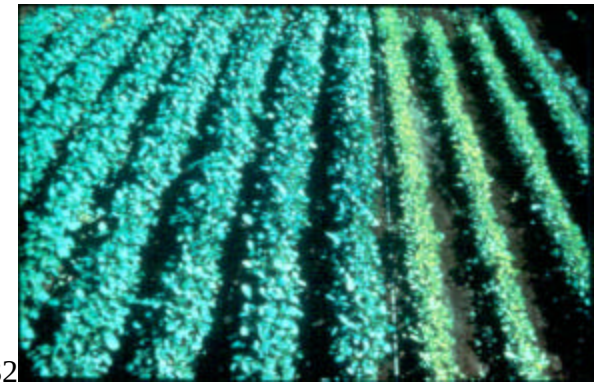
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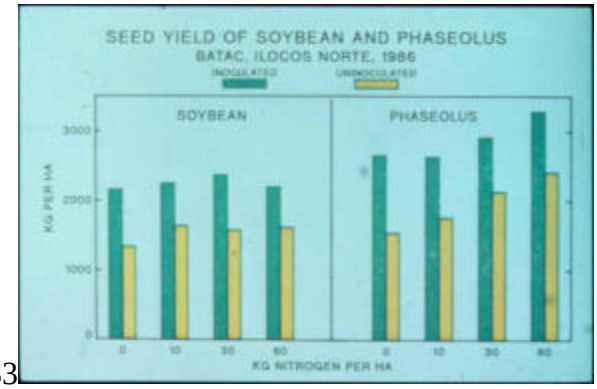
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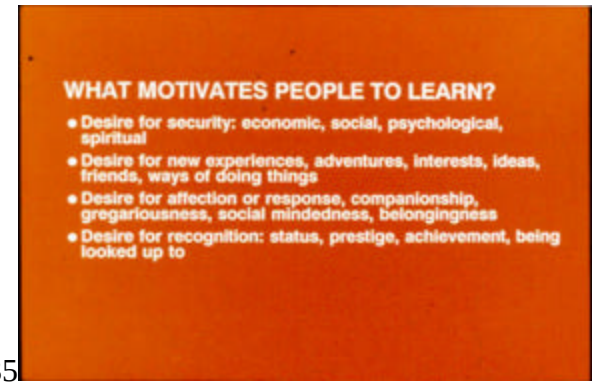
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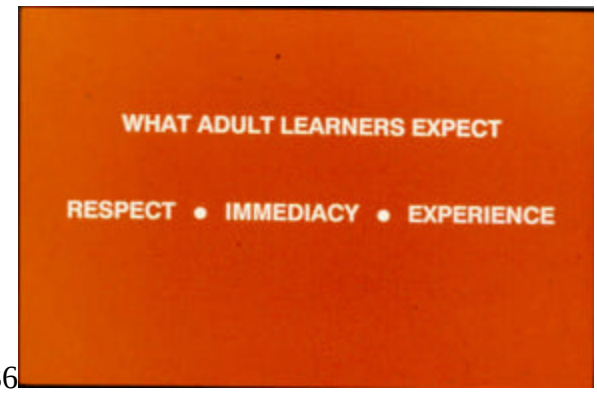
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HOW PEOPLE LEARN

4 Important Requirements To Help Adults Learn Best:

- When they have a strong desire to learn
- When they put into practice what they have learned
- When they have clear objectives or goals
- When they experience satisfaction from what they have learned

38

PEOPLE REMEMBER

- 20% OF WHAT THEY HEAR
- 40% OF WHAT THEY HEAR AND SEE
- 80% OF WHAT THEY DO

39

TEACHING METHOD:

SHOW IT



TELL IT



DO IT



40

WHEN PLANNING,
SYSTEMATICALLY CONSIDER:

WHO
WHY
WHEN
WHAT FOR
WHERE
WHAT
HOW

MODULE 1: DEMONSTRATION 1

DISPLAY OF THE AMOUNTS OF CEREALS AND LEGUMES REQUIRED TO PROVIDE EQUIVALENT AMOUNTS OF PROTEIN

PURPOSE:

- Demonstrate that legumes are high in protein.
- Demonstrate the comparatively large amount of cereal grain that would have to be consumed to obtain an equal amount of protein in much smaller servings of legumes.

CONCEPT OF DEMONSTRATION

This display is a simple reminder that legumes are valuable to the human diet because of their high levels of protein. The high level of protein means a high level of nitrogen, and this is in large part due to biological nitrogen fixation. Even though legumes are rich in protein, it is necessary to eat a mixture of cereals and legumes in order to get all the required amino acids and proteins.

CONDUCTING THE DEMONSTRATION

This demonstration can be carried out easily by displaying the different amounts of cereal and legume seeds that provide the same amount of protein as in a serving of rice. The different seeds should have a label indicating their percent protein.

1. A simple display can be made that shows the different amounts of legumes and cereals needed to provide approximately 30 g protein. The following amounts can be placed in similar (same volume), clear containers with a label including their protein content:

Food Item	Protein	Seed required to provide 30 g protein
	%	g
Rice	7.5	400
Maize	9.5	317
Mungbean	24	125
Peanut	26	114
Cowpea	25	121
Soybean	38	80

The main points are: 1) it takes more rice or maize to get the same amount of total protein as in the legumes; and 2) both cereal and legume proteins are required to obtain complete dietary protein.

2. Some nodulated legumes should be on display during this first review and discussion session. These plants can be removed from the extra pots planted for the cross-inoculation demonstration of **Module 3**. These include lima bean, peanut, soybean and common bean that have been inoculated with their respective, effective rhizobia. The whole plant, including the root system, should be washed clean and then displayed in a water-filled glass container. A few of the large crown nodules on each plant can be sliced open. These plants should be inoculated and sown 30 days before the start of course.

MODULE 1: DEMONSTRATION 2

DISPLAY OF NODULATED LEGUMES

PURPOSE:

- Demonstrate what nodules look like on intact root systems.
- Demonstrate that nodules differ in their appearance depending on the host legume.

CONCEPT OF DEMONSTRATION

This is a simple display of nodules on root systems of different legumes. The legumes chosen should be familiar to the participants. However, this may be the first time participants have seen nodules. Well nodulated root systems are impressive in terms of the amount of mass devoted to nodules. This underscores the significance of BNF in the legume-*Rhizobium* symbiosis. The pink to red color of sliced nodules is provocative. At this point participants learn that the color means an active, effective symbiosis, about which they will learn more in the following modules.

CONDUCTING THE DEMONSTRATION

Clean, washed, well nodulated root systems can be on display at a few stations where small groups of participants can inspect and examine freshly sliced-open nodules. A dissecting microscope or a hand held magnifying lens will aid the examination of the sliced nodule.

Nodulated root systems can be obtained from pot demonstrations, farmers' fields, or wild legumes growing in your region.

MODULE 3: DEMONSTRATION 1

THE CROSS-INOCULATION CONCEPT: LEGUMES REQUIRE SPECIFIC RHIZOBIA

PURPOSE

- Demonstrate that some legume species require different rhizobial species for effective nodulation.
- For effective nodulation, the rhizobia and legume must be properly matched following the "cross-inoculation groups" concept.
- When inoculation is successful and effective, the legume is well nodulated, leaves are green, and nodules, when cut open, are red inside.

CONCEPTS OF DEMONSTRATION

This exercise illustrates that not every rhizobia nodulates all legumes. There is no one type of rhizobia that can be used for all legumes. For example, when the inoculant for soybean seeds are out of supply, do not buy or use inoculants meant for inoculating other legume species. Correct matching of the legume to its recommended inoculant will result in effective nitrogen fixing nodules. When the legume is green and healthy, effective nodules will have developed on the roots. Green plants indicate self-sufficiency in nitrogen. Effective nodules will appear red to pink when cut open.

RECOMMENDATION TO FARMER FROM RESULTS OF THIS DEMONSTRATION

- Always use the correct inoculant for your legume crop.
- To understand what successful inoculation is, the farmer should excavate carefully a few healthy, green plants, look at the nodules, cut open a few and see the red or pink interior.
- Nodules formed by the rhizobia in the inoculant are nourishing his legume crop.
- Nodulation is not a root disease harming his crop.
- Nodules formed by rhizobia have red to pink interiors compared to nodules formed by nematodes.

CONDUCTING THE DEMONSTRATION

It is not necessary to conduct the demonstration to cover the entire range of rhizobial species and their appropriate cross-inoculation groups of legumes as shown in **Table 1** of **Module 3**. Only legumes which are of local or regional economic and agricultural importance need to be selected for the demonstration. The demonstration can be set up in a greenhouse/glasshouse with the legumes grown in potted sand or soil. Greenhouse experiments should be evaluated at 30-35 days.

MATERIALS

Seeds: Soybean (*Glycine max*), bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*) and lima bean (*Phaseolus lunatus*) are used in this exercise. Seeds should have at least a 90% viability rate and be free of insect or mechanical damage. To facilitate inoculation and planting of the seeds at later stages, the seeds need to be mixed in batches and each batch surface sterilized separately. Prepare four batches of seeds, each batch consisting of 20 lima bean, 20 soybean, 20 bean and 20 peanut seeds.

Rhizobia: Obtain peat-based inoculants for soybean (*Bradyrhizobium japonicum*), bean rhizobia (*Rhizobium leguminosarum* biovar *phaseoli*) and cowpea rhizobia (*Bradyrhizobium* sp.).

Potting (growth) medium: Washed and dried river-sand is suitable because it can be steam sterilized (autoclaved) to kill off contaminating rhizobia. Subsoil free of native rhizobia is a better alternative. Soils which are known to have low numbers of ineffective rhizobia can also be used. Sand or soil should be contained in 5 l capacity pots. Ceramic or clay pots can be autoclaved and are preferred for use when sterilizing sand. Soil can also be contained in plastic pots. A total of 24 pots are needed for the exercise.

Sterilization materials: Seed used in this experiment should be surface sterilized to ensure that rhizobia on the surface of the seeds are destroyed. A 2.5% bleach solution (commercial sodium hypochlorite) is needed. Sterile or boiled water must be available. One liter capacity glass containers are required for seed sterilization and inoculation.

PROCEDURE

1) **Potting (growth) medium preparations**: Determine the water-holding capacity of the soil, adjust the soil pH, and add nutrients as described in Demonstration 2 of **Module 7**.

If sand is used it can be autoclaved or heated in the pots to at least 50◦C for 5 hours. Cover the top of the pots with aluminum foil during autoclaving. Keep the sterilized pots in a cool and clean spot in the greenhouse/glasshouse where contamination from insects and airborne rhizobia can be controlled. Pots should be prepared two days before planting.

2) **Seed sterilization and inoculation**: Place each batch of legume seeds in a container. Add sufficient bleach solution to immerse the seeds. Swirl the flask gently and set aside for

2 minutes. Drain off the bleach completely and rinse the seeds with at least eight changes of sterile water. Label the flasks with the strain treatments and an uninoculated control.

3) **Inoculation**: To remove the inoculant(s) from the bag (TAL 182, for example), cut open one corner of the bag with a pair or scissors. (Scissors are sterilized by dipping into a beaker of alcohol and burning off the alcohol with a spirit flame.) Remove a small spoon of inoculant and transfer it to the container of seeds. Swirl the flask until the seeds are coated with the inoculant. Inoculate the rest of the seeds in the flask with the appropriate inoculant, as indicated on the labels. (Remember to sterilize the scissors and spoons before opening the next bag containing a different inoculant.)

4) **Planting the seeds**: For planting the seeds, follow the scheme shown in **Figure 1**. Plant the uninoculated treatments first. Plant three to five seeds of each species per pot. Ensure that seeds are planted at least an inch deep in the soil (or sand). To prevent cross-contamination when planting the inoculated treatments, it is important to disinfect your hands by spraying them with 75% ethanol or washing them with soap and water. Hands and plant accessories must be disinfected between inoculated treatments. Complete planting all the treatments.

5) **Maintaining the potted plants**: To maintain the water-holding capacity of the soil, water the pots with tap water whenever necessary. For pots with soil follow instructions in Demonstration 2, **Module 7**. Water plants grown in potted sand alternately with tap water and half-strength nutrient solution to prevent salt build-up. A nutrient solution formulation is provided in *Methods of Legume-Rhizobium Technology*.

6) **Harvest and recording of data**: Harvest the experiment after 30-35 days of plant growth. Record plant color, nodulation, and plant weight. Cut open nodules to note the color of the interior. Use **Table 1** to record your observations. From the results, analyze the ineffective and effective legume species-inoculant combination.

MODULE 3: DEMONSTRATION 2

GROWTH CHARACTERISTICS OF RHIZOBIA

PURPOSE

- To demonstrate that rhizobia can be broadly classified into two main types based on growth rate and growth reaction on yeast mannitol agar (YMA) medium containing indicators (bromthymol blue and congo red).
- To demonstrate the growth and appearance of rhizobia on YMA from different cross-inoculation groups.
- To demonstrate the growth and appearance of rhizobia cultured in liquid yeast-mannitol (YM) medium.

CONCEPTS FROM DEMONSTRATION

The two main classes of rhizobia are:

- fast-growing rhizobia with an acid reaction (bromthymol blue indicator turns yellow)
- slow-growing rhizobia with an alkaline reaction (bromthymol blue indicator turns blue)

Rhizobia show little or no congo red adsorption and appear faintly pink to white. Nonrhizobia absorb congo red and appear as red colonies.

Rhizobia that are fast-growing are from the alfalfa, pea, bean, clover and the Leucaena groups of legumes. Rhizobia that are slow-growing are found in the soybean and cowpea groups of legumes.

Rhizobia from the various cross-inoculation groups appear similar on YMA or YM medium and host legumes from which they were isolated cannot be determined from their growth characteristics.

Recommendations to farmers from results of this demonstration

Knowledge on the growth characteristics of rhizobia is not essential to the farmer. Demonstration of growth characteristics of rhizobia is useful knowledge to the extension agent. When discussing legume BNF with researchers and inoculant producers, it is important that regional extension workers with expertise in BNF know some characteristics of rhizobia.

CONDUCTING THE DEMONSTRATION

Pure cultures of rhizobia from different cross-inoculation groups are needed. This demonstration requires the assistance of a microbiologist with experience working with rhizobia. The demonstration can only be set up if laboratory facilities are available for sterile media preparation and equipment for performing sterile or aseptic work. See *Methods in Legume Rhizobum Technology* for procedures.

MODULE 4: DEMONSTRATION 1

DEMONSTRATING DIFFERENCES IN EFFECTIVENESS OF STRAINS OF RHIZOBIA

PURPOSE:

Demonstrate that strains of rhizobia differ in their ability to fix nitrogen as measured by benefit to the legume host.

Demonstrate that strain effectiveness can be based on evaluation of several factors.

CONCEPTS OF DEMONSTRATION

This exercise demonstrates the range of effectiveness found among the soybean rhizobia. Effectiveness is defined as the amount of N_2 fixed by a particular strain of rhizobia under the existing conditions. The most important manifestation of effectiveness is in the response of the plant, as indicated by its size and color. Differences between strains may also be found in comparing their nodule number and weight, location on the root system, and the interior color of nodules. When soil N is low (and other factors are not limiting) the amount of N_2 fixed is a function of the effectiveness of the strain in the nodules. While soil may contain a mixture of strains that vary in effectiveness, strains in inoculants should all be very effective.

FARMER RECOMMENDATION FROM RESULTS OF THIS DEMONSTRATION

Farmers should inoculate their legume crops to ensure obtaining the most effective rhizobia.

Farmers can estimate the effectiveness of rhizobia in their field by some simple examinations of the nodulated legume.

CONDUCTING THE DEMONSTRATION

Two sets of treatments will be available for use in the demonstration, one from soil and one from artificial media. If the set from soil does not show the response, the set from artificial media can be used in its place. In small groups the participants should examine the plants and record the observations on the attached worksheet. After data from all the groups has been compiled, a discussion can be held on the criteria for evaluating

effectiveness.

Growth media: Soybean-rhizobia free soil and/or Leonard jars. (See Methods in Legume-Rhizobium Technology) The demonstration in soil (if successful) is more realistic. The Leonard jar trials can be used as a backup if the soil trial is not successful. Follow suggestions in Module 3, Demonstration2, about conducting a demonstation with potted soil.

Rhizobia: SM5, USDA 110, USDA 123, USDA 33. Controls should include an uninoculated or a non-nodulating soybean line labeled 'uninoculated.' These strains and soybean seed can be obtained from NifTAL.

Soybean: Choose a line adapted to prevailing daylength and growth conditions of the greenhouse.

Duration: 35-42 days from emergence.

Replicates: 5 (4 for harvest in demonstration; 1 extra for evaluation of soil trial1-2 days before demonstration).

Observations: In teams of 4 each, the following observations and determinations should be recorded on the attached data sheet:

ranking of treatments by size;

number of sets of trifoliate leaves;

color of foliage; green, light green, yellow;

fresh weight of entire plant;

location of nodules; crown, tap or laterals of root system;

number of nodules;

fresh weight of nodules;

color of nodule cross-section based upon observation of 5 randomly selected nodules.

GENERAL PROCEDURE

A soil low in available N and free of soybean rhizobia should allow the demonstration of effectiveness of these strains. Their effectiveness under such conditions should be 110> 123 > 33> SM5 (ineffective) = uninoculated control.

If it is questionable whether the soil is free of soybean-rhizobia, then a non-nodulating soybean line is suggested as a replacement for the uninoculated treatment. The safest

approach would be to include both of these controls, and decide which to use in the demonstration upon examination. If the entire soil trial appears worthless due to high N or indigenous rhizobia, the Leonard jar trial can be harvested instead during the final demonstration.

After the observations and determinations have been recorded by each team, the data from all teams can be listed on a chalkboard. Volunteered interpretation of the overall data is encouraged. If not brought up in discussion, the following points may be raised:

Is any single criterion best for evaluating effectiveness (e.g., nodule number)?

Which of these criteria can be easily used by an extension agent in the field or a farmerto estimate effectiveness on a given legume?

MODULE 4: DEMONSTRATION 1

WORKSHEET

	Strains				
	Uninoculated	SM5	USDA33	USDA123	USDA110
Plant					
Number of Trifoliate Sets					
Color of Plant					
Fresh Weight of Whole Plant					
Location of Nodules					
Number of Nodules					
Fresh Weight of Nodules					
Color of Nodule Cross-section					

MODULE 4: DEMONSTRATION 2

ESTIMATING NITROGEN INPUTS OF A SOYBEAN CROP

PURPOSE:

Demonstrate that nitrogen inputs (from soil and BNF) for a legume crop can be reasonable estimates from available data or information.

Demonstrate that the high protein (and therefore nitrogen) content of legumes requires higher amounts of N from BNF with increasing yields.

CONCEPTS OF DEMONSTRATION

This exercise demonstrates the utility of using some simple calculations to estimate N inputs of a soybean crop. Participants predict N needs based on available information, including what they know from a non-legume grown in their area. The important concept that N may be supplied from soil and from BNF is reinforced.

CONDUCTING THE DEMONSTRATION

This exercise can be divided in two parts; part one asks participants to estimate the rate of nitrogen supply from the soil needed to obtain an average (yield and % N) soybean crop, and part two asks them to estimate the increasing amounts of N required from BNF to obtain increased soybean yields.

Part one

According to Agricultural Statistics 1985 (U'.S.D.A., U.S. Gov't Printing Office, Wash., D.C.) the average yield of maize in Indonesia was 1800 kg/ha. With a normal protein content of 9.5%, and therefore a nitrogen content of 1.52% (%N = Protein/6.25), the participants can calculate the N yield and the daily N supply rate from the soil.

Assuming that like most soils N is the most limiting nutrient, and other factors are not yield limiting, consider the introduction of a soybean crop. If an average yield (1000 kg/ha) and 6.08% nitrogen content (based on 38% protein in seed) is expected what will be the N yield, and the N supply rate from the soil required to meet the soybean crop N requirement?

The following table should be constructed:

Crop	Estimated Yield	Nitrogen Content	Nitrogen Yield	Crop Duration	Nitrogen supply rate required
	kg/ha	%	kg/ha	days	kg/ha/day
Maize	1800	1.52	27	120	0.23
Soybean	1000	6.08	61	95	0.64

Shaded figures to be calculated, others given.

Two important and related questions arise here: 1) Do the participants think their soils can meet the higher N supply rate required for this soybean crop?, and 2) if not, what lower yield would be expected for soybean?

Part Two

From Part 1, it is shown that a 1000 kg/ha soybean crop has a N yield of 61 kg/ha. Let us assume that the 27 kg of N/ha found in the maize crop is the nitrogen supplying capacity of an average Indonesian soil. The participants can then calculate the amount of N that must be supplied from BNF to make up the difference. The percent N from BNF can then be calculated. Once this is done, new estimates of N yield, amount and percent N from BNF, can be calculated for hypothetical higher yields.

The following table should be constructed:

Estimated Soybean Yield	Nitrogen Yield	Nitrogen from soil	Nitrogen from BNF	%N from BNF
kg/ha	kg/ha	kg/ha	kg/ha	%
1000	61	27	34	55
1500	91	27	64	70
2000	122	27	95	78

Discussion on the implications of this data is encouraged. It can be seen that the average soybean yield (1000 kg/ha) will require about 55% of its N from BNF.

Higher yields will require higher levels and percentage of N from BNF.

This exercise can be conducted in teams or with the whole group. In either case, a master table for parts 1 and 2 can be constructed on a chalkboard, and the calculated figures filled by consensus.

WORKSHEET

ESTIMATING NITROGEN INPUTS OF A SOYBEAN CROP

1. The average 1985 yield of maize in Indonesia was 1800 kglha. The normal protein content is 9.5%. Since protein is 6.25% nitrogen, the nitrogen content of the harvested maize is 1.52% (9.5 divided by 6.25). With this information you can calculate the nitrogen yield and an average nitrogen supply rate from an unfertilized soil. Fill in these two figures for maize in the table below.

Crop	Estimated Yield	Nitrogen Content	Nitrogen Yield	Crop Duration	Nitrogen Supply Rate
	kg/ha	%	kg/ha	days	kg/ha/day
Maize	1800	1.52	-	120	-
Soybean	1000	6.08	-	95	-

Now consider a soybean crop that will be growing under the same conditions. The anticipated yield is 1000 kg/ha. With a typical protein content of 38%, this is a nitrogen content of 6.08%. As with the maize, calculate the nitrogen yield and the required N supply rate from the soil (assuming that all the N came from the soil). Fill in these figures in the table.

Despite yielding lower than maize, the N yield for soybean is much higher. Why is this? Do you think most soils on which maize is grown could increase their N supply rate to meet the demand in the soybean?

2. Let us assume that the N yield of the maize (part 1) represents the N supplying limit of a given soil. Assuming further that the typical N content of soybean seed is 6.08% (part 1) it is obvious that BNF must supply some of the crop's N. For the typical 1000 kg/ha yield of soybean, how much N in this case will have to be supplied from BNF? What percent of the total N is this? Using the same assumptions, calculate the N yield, N from BNF, and % N from BNF for cases where higher yield, 1500 and 2000 kg/ha, were obtained. Complete the following table.

Estimated Soybean Yield	Nitrogen Yield	Nitrogen from soil	Nitrogen from BNF	%N from BNF
kg/ha	kg/ha	kg/ha	kg/ha	%
1000	-	27	-	-
1500	-	27	-	-
2000	-	27	-	-

Discuss the implications of these figures with your colleagues and instructors.

MODULE 5: DEMONSTRATION 1

LABORATORY SCALE INOCULANT PRODUCTION

PURPOSE

- To give the extension worker a basic understanding of inoculant production.

CONCEPTS OF DEMONSTRATION

Rhizobia cultures must be handled under strict aseptic conditions.

A fermentor is a vessel in which medium growing rhizobia can be sterilized and which has provisions for the aseptic inoculation of starter cultures and aeration using protected filters.

Rhizobia are mass cultured in a fermentor containing a sterile growth medium and supplied with a flow of sterile air.

At a temperature of 26◦C and a starter culture of 1% of the fermentor broth volume, bean rhizobia will require approximately 3 days to reach maturity or a count of 10^9 rhizobia per ml of culture broth. Soybean rhizobia will require approximately 5 days.

After the fermentor culture has reached maturity it is injected into a pregerminated sterile peat carrier for the production of a pure culture inoculant.

RECOMMENDATIONS TO FARMERS FROM RESULTS OF THIS DEMONSTRATION

Knowledge of inoculant production is not essential to farmers. Extension agents should have a basic understanding of the concept. They are advised to tell farmers that inoculant production requires personnel with specialized skills and special equipment.

CONDUCTING THE DEMONSTRATION

See *Methods of Legume-Rhizobium Technology* for the details of assembling a small glass fermentor, p. 206-215.

This demonstration requires an instructor who is a microbiologist with experience in working with rhizobia. The instructor will assemble a simple glass fermentor and inoculate another fermentor already assembled and sterilized. He will harvest full grown cultures from a third fermentor and inject it into a bag of presterilized peat carrier.

MODULE 5: DEMONSTRATION 2

QUALITY CONTROL IN INOCULANT PRODUCTION: FERMENTOR BROTH

PURPOSE

- To demonstrate the procedures required to assure purity of culture during production of inoculum in fermentors.

CONCEPTS OF DEMONSTRATION

Rhizobia must show the proper reactions when streaked on test media (**Module 3**, Demonstration 2).

Odor, color and pH can also be indicators of purity.

A pure rhizobia culture must be gram negative.

A pure culture of rhizobia must agglutinate with an antiserum produced against it.

RECOMMENDATIONS FOR FARMERS FROM RESULTS OF THIS DEMONSTRATION

Knowledge of quality control in inoculant is not useful to farmers. It is useful to extension agents for a better understanding of how inoculant is produced and how the quality of inoculant is verified.

CONDUCTING THE DEMONSTRATION

Material Requirements:

fully assembled and operational glass fermentor
10 ml plastic syringe with 23 g needle
test tube rack with three 10 ml test tubes
antiserum for strain used in fermentor
bottle with saline solution (0.85% NaCl)
1 ml pipette
tube of preagglutinated culture
set of gram stain solutions
microscope slides
cover slips
prepared gram stain of TAL 102

preadjusted microscope

solution of bromthymol blue (BTB)

yeast mannitol agar (YMA) plate containing bromthymol blue with pure culture of *Bradyrhizobium* TAL 102 growing on it

YMA plate containing congo red (CR) with pure culture of *Bradyrhizobium* TAL 102 growing on it.

A microbiologist familiar with rhizobia is required to conduct this demonstration. The instructor will perform quality control methods while narrating his demonstrations which will consist of the following activities:

1) Show the YMA plates containing CR and BTB and point out that the culture of TAL 102 had been streaked onto these media as a purity test prior to use for mass culturing.
2) Draw fermentor culture broth aseptically and place into a test tube. Explain color and smell.
3) Take one ml of culture into another test tube for pH test.
4) Put 1 ml and add antiserum and saline for agglutination test. Sow preagglutinated culture in the last tube.
5) Make a smear on a microscope slide. Under the microscope, show the slide prestained with gram stain.

MODULE 5: DEMONSTRATION 3

INFLUENCE OF STORAGE CONDITIONS ON TEMPERATURE OF STORED INOCULANT

PURPOSE

- Legume inoculant consists of living rhizobia which can be killed by exposure to the sun or high storage temperatures. The purpose of this demonstration is to show that suitable storage temperature can be achieved, even in a hot climate, by taking special precautions.

CONCEPTS OF DEMONSTRATION

Inoculant should not be exposed to the sun or stored in a hot shed.

Inoculant may be wrapped in a moist towel or placed in a basket covered with a wet towel to keep it cool and sheltered from the sun.

A ceramic urn buried at a shady spot can keep inoculant safe and cool.

RECOMMENDATION TO FARMERS FROM RESULTS OF THE DEMONSTRATION

Always keep rhizobia away from sun and heat.

Make use of the storage suggestions shown in the demonstration or improvise your own methods.

When in doubt, use a thermometer to verify temperatures.

In the field, inoculate in the shade. When planting seeds, cover seeds with soil soon after sowing.

Inoculants must be kept in closed bags.

CONDUCTING THE DEMONSTRATION

Explain to the farmer that inoculants cannot be treated like fertilizer when transporting and storing them. Convince him that sunshine is not good for them. Storage in a hot shed must be avoided.

Make a small demonstration to show that it is possible to achieve lower temperatures by simple means.

Materials Requirements:

a sunny spot
a hot storage shed
two wet towels
four thermometers
one ceramic container with lid (8 liter or more capacity)
one digging tool
eight 10 g bags of inoculant

Place two bags of inoculant in the following locations:

1) Directly into the sun. A spot should be chosen which will remain unshaded for at least 1 hour.
2) Into a hot storage shed.
3) Into a basket after wrapping the inoculant in a moist towel. Cover the basket with another moist towel.
4) In a ceramic vessel buried in the soil in a shady spot. A thick wooden lid should cover the vessel.

Add a thermometer to each one of the four storage treatments and take readings after 1 hour.

Record the temperatures reached by the inoculant.

MODULE 5: DEMONSTRATION 4

QUALITY CONTROL IN INOCULANTS

PURPOSE

- To demonstrate the procedures used for determining the contents of viable rhizobia in sterile and nonsterile peat-based inoculants.

CONCEPTS OF DEMONSTRATION

The quality of an inoculant depends on the number of live and infective rhizobia in it. Enumeration methods require that the inoculant be diluted serially. Several dilutions are then selected for counting. For inoculants based on sterile carriers, aliquots of these dilutions can be spread onto plates containing solid growth medium. The resulting rhizobia colonies can then be counted.

For inoculants based on nonsterile carriers, this method is not practical because other microorganisms present interfere with the plate count. Aliquots of the serial dilution are therefore pipetted onto the roots of seedlings which have been grown aseptically. The nodulation ability of these dilutions will then give information for an estimate of the number of rhizobia present.

RECOMMENDATIONS TO FARMERS FROM RESULTS OF THIS DEMONSTRATION

Knowledge of inoculant quality control procedures is not useful to farmers. It is useful to extension agents to further their basic understanding of inoculants and aid them when discussing distribution and use of inoculant with the inoculant producer.

CONDUCTING THE DEMONSTRATION

An instructor with knowledge of the microbiology of rhizobia is required. See *Methods in Legume-Rhizobium Technology* for details on plate counts and plant infection tests (MPN) to enumerate rhizobia.

Material Requirements:

growth room or chamber

dilution series of inoculant in test tube

a plant infection test with the appropriate species already set up with replications from 10^{-5} to 10^{-11}. This test is based on a high quality inoculant. Inoculant below 1 X 10^{6} rhizobia/g is not useful, therefore, the dilution series does not have to begin until

10^{-5}.

a plant infection test based on a low quality inoculant to simulate exposure to heat. Dilution series in duplicates from 10^{-1} to 10^{-6}. Highest nodulated replication 10^{-5}.

duplicate spread plates showing emergency colonies as a result of 10^{-7} dilution.

Demonstration: The instructor will narrate and explain while showing the following:

1) a dilution series of a peat-based inoculant in test tubes.
2) a completed plant infection test of a high quality inoculant on soybeans in growth pouches.
3) a completed plant infection test of a low quality inoculant on soybean in growth pouches.
4) a completed plate count on YMA in petri dishes.
5) the instructor will briefly discuss the evaluation of the plant infection count and the plate count.

MODULE 5: DEMONSTRATION 5

SEED INOCULATION

PURPOSE

- To demonstrate the preparation of stickers, methods of coating seeds with inoculant and a seed pelleting technique.

CONCEPTS OF DEMONSTRATION

Sticker materials are recommended to bind the rhizobia to the seed. The stickers used in the following demonstrations are gum arabic, carboxymethylcellulose, and sugar. All these adhesives, must be dissolved in water before use.

Two seed coating methods are used; the slurry method and the two-step method. In the slurry method, inoculant is first mixed with the sticker. The resulting slurry is then applied to the seeds.

The two-step method requires seed coating in two stages. First, the seeds are coated with a sticker. The inoculant is then added and coated onto the sticky seeds. The amounts of sticker used for each method vary with seed size (**Module 5**).

Under certain conditions (**Module 5**) it is advisable to pellet inoculated seeds with a protective layer of powdered calcium carbonate or rock phosphate. This treatment is most commonly done with seeds of pasture legumes. The pellet is applied after seed coating by either the slurry method or the two-step method. The seeds are rolled in the pelleting material immediately after inoculation while they are still wet and sticky.

RECOMMENDATIONS TO FARMERS FROM RESULTS OF THIS DEMONSTRATION

To learn proper seed inoculation techniques

To follow the demonstrated procedures for seed pelleting when required.

To use stickers for seed inoculation.

CONDUCTING THE DEMONSTRATION

The amounts of materials needed should be gauged according to the number of participants in the demonstration exercise. The list of materials below is based on 10-20 practicing participants.

Material Requirements:

balance
heating plate
source of clean water
measuring container (100-500 ml capacity)
plastic bags of 1 liter capacity
plastic bags, very strong, of 20 liter capacity
wooden stirring rods
plastic buckets, 20-25 liter (5-6 gallon) capacity, with lids
methylethylcellulose (100g)
gum arabic, granular (100 g)
sugar, granular (1kg)
calcium carbonate, powdered in 1 kg
soybean inoculant, 100 packages
seeds of soybean
seeds of a pasture legume

Preparations just prior to demonstration exercise:

Twenty batches of 100 g soybean seeds in 1 liter plastic bags

Twenty batches of 100 g other seeds in 1 liter plastic bags

Ten batches of 5 kg soybean seeds in 20 liter plastic bags

Two batches of 4 g carboxymethylcellulose, approximately 250 ml

Two batches of 40 g gum arabic, approximately 250 ml

Two batches of 500 g sugar in 2 liter container

Twenty batches of 2 g inoculant (protected from moisture loss)

Twenty batches of 1.5 g inoculant (protected from moisture loss)

Ten packages of 50 g inoculant (protected from moisture loss for bulk coating)

Twenty batches of 35 g powdered calcium carbonate

Note: The techniques of this demonstration should be taught through participation. First demonstrate, then have the important parts of your demonstrations repeated. Make sure to correct any mistakes your participants may make. In order to save materials, seed batches are small for this demonstration. They may, of course, be modified according to materials available and number of people participating.

Measurements are given in grams, liters and milliliters. You are advised to convert these specific volumes and weight measurements into more convenient local units. One teaspoon, for instance, holds 5 ml of sticker and 1 heaped teaspoon of inoculant is 5 grams. Three teaspoons make 1 tablespoon. If these measurements do not apply, other measuring utensils that are readily available may be used (eg., tins, jars, etc.).

Several stickers are used here for comparison. Demonstrations for farmers may use readily available sticker (e.g., sugar).

Preparing Sticker Materials

Gum arabic. Heat 100 ml water in a container. Add 1 teaspoon of gum arabic and stir until it is dissolved. In the same manner, add the remaining gum while stirring until the total of 40 grams are dissolved. Set aside to cool.

Carboxymethylcellulose. Dissolve 4 g in 100 ml of cool water. Stir until the cellulose powder is dissolved.

Sugar. Place 100 ml of water into a small pot or beaker. Add 10 grams of sugar. Stir until dissolved.

THE SLURRY METHOD

Preparing the slurry. For coating soybean seed, a slurry consisting of one part of inoculant and three parts sticker is recommended. Refer to **Module 5** for the proper proportions for seeds of various sizes. For demonstration and practice of this procedure, only a small amount of seed will be coated.

1) Weigh 2 g of inoculant and place it into a container. Add 6 ml of water. Mix the inoculant and the water until a uniform mixture is achieved.

2) Weigh 100 g of seeds and place them into a container. Add 2 ml of the slurry. Stir the seeds with a wooden stick until they are uniformly coated with the inoculant slurry. Alternatively, the seeds may be coated by shaking as described for the two-step method below.

3) Immediately after coating, spread the seeds onto clean paper and allow them to dry.

Repeat the seed coating procedure (Steps 1-3) with slurries made from the other sticker solutions to achieve the treatments as summarized below:

a) 100 g of soybean seeds coated with 2 ml of a slurry, prepared by mixing 2 g of inoculant with 6 ml of gum arabic solution.

b) 100 g of soybean seeds coated with 2 ml of a slurry prepared by mixing 2 g inoculant with 6 ml of carboxymethylcellulose solution.

c) 100 g of soybean seeds coated with 2 ml of a slurry prepared by mixing 2 g inoculant with 6 ml of sugar solution.

After coating, compare the four different treatments. Inspect them for evenness of coating and for adhesion quality. The best coating is usually achieved with gum arabic followed closely by carboxymethylcellulose as a sticker. Sugar should be third best. Water looks good initially but the inoculant tends to flake off the seed after drying. Whenever possible, a

sticker should be used for seed coating.

THE "TWO-STEP" METHOD

Place 100 g of soybean seeds into a plastic bag and add 2 ml sugar solution. Inflate the bag and gather the ends of the plastic bag and twist it shut, trapping a maximum of air inside. The walls of the bag must be tight. Shake the bag vigorously for about one minute until the seeds are uniformly coated. Open the bag and add 1.5 g of inoculant. Close the bag and shake gently. Stop after one minute because prolonged shaking could dislodge the inoculant from the seeds.

Repeat the coating procedure with the following treatments:

1) 100 g seed wetted with 1.5 ml gum arabic solution and then coated with 1.5 g peat inoculant.
2) 100 g seed wetted with 2 ml carboxymethylcellulose and then coated with 1.5 g peat inoculant.
3) 100 g seed wetted with 2.5 ml water and then coated with 1.5 g peat inoculant.

Immediately after coating, spread the seeds on paper and allow them to dry in a cool, shady place. Compare the five different treatments. When we compare them with the slurry treatments we will immediately notice a darker color on all the seeds. We have actually applied more inoculant to the seed by this method. Again, the gum arabic and the carboxymethylcellulose treatments usually look better followed by the sugar treatments. The inoculant will not stay on as well when water is used.

The two-step method allows us to apply a maximum amount of inoculant to the seed. We could actually apply much more inoculant, especially with gum arabic and carboxymethylcellulose. If we used, for instance, 3 ml of the sticker, we could coat as much as 10 g of inoculant for 100 g seeds, which means 10^8 rhizobia per seed if the inoculant contains 10^9 rhizobia per gram. Such a rate is, under normal conditions,rarely desirable and not very cost effective for farmers. To apply more than this is not practical because the seeds would clump if more sticker than 3 ml of sticker per 100 g of soybean seeds were applied.

INOCULATING FOR FIELD APPLICATION

If sturdy plastic bags can be obtained, the upper limit for coating by shaking sticker-coated seeds with inoculants may be 5 kg of seeds. The inflated bag should then be rolled on the ground for seed coating. A better container may be a 20 liter plastic bucket with a lid. The sticker most likely on hand may be sugar. If available, carboxymethylcellulose or gum arabic may be used instead.

Procedure. Weigh 5 kg of soybean seeds and place them into a container of 20 liter capacity. Add 220 ml of sugar solution; close the lid tightly and shake for 1 minute. Open the container and make certain the seeds are evenly coated, not clumped together, and that no sticker residue is clinging to the walls. Add 50 g of inoculant and tightly close the lid. Shake gently. After 1 minute open the lid and inspect seeds for uniformity of coating. If coating is not complete, immediately continue shaking for 30 seconds. After coating, spread seeds on a clean canvas or paper. After the seeds have dried, package them and place them into a cool, shaded place until sowing. Sow as soon as possible after coating.

If more than 5 kg need to be coated and the container gets too heavy for shaking by hand, it can be rolled on the ground.

Pelleting Seeds

Pelleting after slurry application. Make a slurry from the 4 ml of sugar solution and 5 g of inoculant. Place 100 g of pasture legume seeds in a plastic bag of 1 liter capacity. Add 4.5 ml of the slurry. Close the bag and trap as much air inside as possible. Shake until the seeds are uniformly coated. Open the bag and add 35 g of calcium carbonate powder. Shake gently until all the seeds are uniformly pelleted. Spread pelleted seeds on paper and allow them to dry in the shade.

Repeat the application with 4.5 ml slurry with gum arabic as a sticker.

Pelleting after the "two-step method of inoculation." Place 100 g of siratro seeds into a plastic bag of 1 liter capacity. Add 4 ml of sugar sticker. Close bag with air trapped inside. Shake until coating has been achieved. Add 1.5 g of inoculant and shake gently for 1 minute. Open the bag and add 35 g of calcium carbonate. Shake gently until all seeds are uniformly coated. Spread pelleted seeds on paper and allow to dry in the shade.

Repeat this treatment with 4 ml of gum arabic as a sticker.
Compare all four treatments for evenness of coating, firmness of pellet and amount of calcium carbonate adhering to the seed.

To accommodate the pelleting material, more sticker must be applied. Carboxymethylcellulose may also be used as a sticker. Water is unsuitable for pelleting because it does not make a firm enough pellet.

MODULE 5: DEMONSTRATION 6

SOIL INOCULATION

PURPOSE

- To familiarize extension workers and farmers with methods of soil inoculation. The techniques described do not make use of special soil inoculant. Instead, the methods shown make use of the more readily available peat-based seed inoculant and convert it for soil application.

CONCEPTS OF DEMONSTRATION

There are two ways of bringing the inoculant in contact with the seed. The seed can be inoculated with rhizobia and then placed into the soil, or the soil can be inoculated before sowing. The latter method, though less common, is advisable under certain conditions (**Module 5**).

There are two ways in which seed inoculant may be used for soil application.

1) **The dry method**. Inoculant is first diluted with silica sand or soil and then applied to the soil.

2) **The wet method**. Seed inoculant is diluted in water and then applied to the furrow.

RECOMMENDATIONS TO FARMERS FROM RESULTS OF THIS DEMONSTRATION

Use soil inoculation when soil conditions or seed treatment may kill rhizobia in seed applied inoculant or inoculation at very high rates/ha are required.

CONDUCTING THE DEMONSTRATION

Find a cooperating farmer and ask him to prepare a small field area for the demonstration. Try to get farmers of the region together as participants in the demonstration. The following materials are needed:

A field plot with 12 furrows of 10 m each (This field plot should be located near the laboratory facilities).

One simple balance

5 kg of fine silica sand

One wooden stirring stick

One graduated 2 liter vessel (as shown in Fig. 5-9, pg. 5-20).

One empty bucket with tight lid

Two 100 g packages of seed inoculant

Preparations just prior to demonstration exercise:

20 batches of 10 g inoculant in small covered beakers

Note: The rate of application depends on the quality of the inoculant and soil conditions. A minimum of 1.6 kg inoculant per ha are needed if the inoculant contains at least 10^9 rhizobia per gram.

Dry application. This method is useful for inoculating moist soil. Place 1 kg of dry sand or fine dry soil into a bucket. Add 10 g inoculant. Close the lid tightly and shake the bucket by hand or roll it on the ground until inoculant and sand are thoroughly mixed. Open the bucket and inspect the mixture for uniformity. Continue mixing if required.

Distribute the diluted inoculant in a band over 100 m of furrow.

Plant the seeds immediately after inoculation. Close the furrow shortly after sowing to protect the inoculant from sun and heat. Irrigate after planting if possible.

Wet application. This method is especially useful in dry soil.

Measure 10 liter of water into an applicator vessel as shown in **Figure D5/6-1**. Add 10 g inoculant and mix thoroughly. With the outflow preadjusted to the desired flow rate, point the applicator tip into the furrow and dispense an even flow of liquid over 100 m. For uniform application, it may be advisable to make several passes with a reduced rate of flow.

Here again, sow immediately after inoculation and cover the furrow as soon as possible. Irrigation is always advisable after inoculation.

MODULE 5: DEMONSTRATION 7

EVALUATING THE QUALITY AND EFFECTIVENESS OF INOCULANTS

Purpose

- To provide the extension agent with a simple method for evaluating the quality of inoculants.

Concepts of Demonstration

The quality of inoculants can vary greatly. A test lab for instance, reported that some producers in it's country did not even have rhizobia in their products. Frequently, the extension agent and also the inoculant user are uncertain about the quality of inoculants offered for sale. Without access to a quality control laboratory, the extension agent is at a loss unless he can perform a quality assessment himself.

Inoculants can be tested by assessing their ability to nodulate legumes effectively. This can be done by the "grow-out" test. In this test, the inoculants in question are used to inoculate their specific host.

Recommendations to Farmers from Results of this Demonstration

The extension agent can use the grow-out test to identify manufacturers of quality inoculants. He can then assure farmers that the inoculant they purchase is good quality.

Conducting the Demonstration

Pots; minimum size 2 liter capacity.

Soil must be free of rhizobia which will nodulate the legume of interest. If not available, another media such as silica sand can be used.

A protected facility to set up the pots. A greenhouse would be ideal. A bench in a nursery-type greenhouse would be second best. A bench in a wind protected area outside may also suffice.

A watering can or hose.

Clean water.

The inoculants to be tested.

Legume seeds compatible with the inoculant.

Sugar or other sticker material. Make the appropriate sticker solution.

Plastic bags.

Setting up the experiment (refer to **Module 7**, Demonstration 2, for some information on setting up a pot test with soil, and **Module 3**, Demonstration 1, for suggestions using sand or a growing media.)

Coat 50 g portions of seeds by the sticker method with each of the test inoculants.

Set up four pots for each inoculant to be tested. The soil used should usually have the fertilizer amendments already added (except nitrogen see **Module 7**, Demonstration 2). In addition, set up four pots for uninoculated control and another four pots as +N control. These pots should have the full fertilizer amendment including nitrogen.

If you use sand or a media, a complete nutrient (N-free) solution must be added. See *Methods in Legume-Rhizobium Technology* for a formula.

If, for instance, you have three inoculants to be tested you will have five treatments including one uninoculated control and one control to which nitrogen has been added; a total of 28 pots as shown below:

Inoculant Producer	Number of Pots (replications)			
1	■	■	■	■
2	■	■	■	■
3	■	■	■	■
Uninoculated	■	■	■	■
+ N uninoculated	■	■	■	■

First, plant the uninoculated seeds. Plant six seeds, well distributed, in each of the eight pots of your control treatments.

Similarly, sow the seeds of all your inoculated treatments; six seeds/pot. Avoid cross-contamination between pots. Wash your hands after each treatment. Water regularly.

After 10 days, thin plants to three per pot. Do not pull out the plants but cut them at the soil surface. The plants remaining should be the most uniform and healthy looking in each pot.

Evaluating the results

After 4 weeks inspect plants. Well nodulated plants should be green and have growth similar to the nitrogen control.

Carefully remove plants from the pots and inspect the root systems. Remove the tops and weigh, if possible. In the case of soybeans, a good inoculant should produce a healthy, well nodulated plant with approximately 20 or more nodules. When cut in half, the nodules should be red or pink inside. The weight of the tops should be similar to that of the nitrogen control. Count the nodules on the plants of each treatment and calculate the mean nodule number per treatment. Tabulate the results and compare the nodules obtained from each inoculant treatment.

The non-inoculated plants in the control treatments should be smaller and yellowish. Ideally, there should be no nodulation. Nodulation may occur if there are native rhizobia in the soil. Most often these nodules are ineffective and white on the inside when cut open.

If the non nodulated controls are healthy and well nodulated and do not differ much from the inoculant treatments, the soil chosen has effective native rhizobia and inoculation is not necessary.

MODULE 6: DEMONSTRATION 1

THE EFFECT OF NITROGEN FERTILIZER AND MANAGEMENT ON NODULATION AND GROWTH OF LEGUMES

PURPOSE

- Show how mineral nitrogen in soil influences nodulation of legumes.
- Demonstrate that nitrogen in the soil eliminate the differences in plant growth due to rhizobia.
- Demonstrate the effect that management has on nitrogen requirements of legumes and BNF.

CONCEPTS OF THE DEMONSTRATION

Modules 4 and **6** discuss the role of mineral nitrogen on legume BNF. Legumes form an effective symbiosis with rhizobia when the plants need for nitrogen is not met by the nitrogen in the soil. When there is abundant nitrogen in the soil from mineralized organic matter (**Module 1**) or from fertilizer, there may be no nodulation on legumes, even if they are inoculated with effective rhizobia. Legumes prefer to use available mineral nitrogen rather than form nodules and fix atmospheric nitrogen with rhizobia.

Whether legumes form nodules and fix nitrogen is dependent on the balance between the supply of mineral nitrogen in the soil and the nitrogen requirement of the legume. When there is less mineral nitrogen in the soil available than the plant requires, and there are effective rhizobia in the soil, the legume crop will fix nitrogen. If management affects the nitrogen requirement of the legume, nodulation and BNF will also be affected. Poor management reduces the nitrogen required by the plant for growth and therefore reduces BNF. It is important for extension agents and farmers to realize that good management will increase BNF.

FARMER RECOMMENDATIONS FROM RESULTS OF THIS DEMONSTRATION

Farmers should avoid applying fertilizer nitrogen to legume crops since it may reduce the benefit they obtain from BNF.

Although fertilizer nitrogen produces healthy legume crops, the farmer may lose money by applying fertilizer nitrogen rather than inoculating with effective rhizobia.

The benefit that farmers obtain from BNF increases when management can increase growth and yield of the legume crop.

CONDUCTING THE DEMONSTRATION

Soybean is usually good for this demonstration. Soybean rhizobia are not widely distributed in the tropics which will make the differences in strain effectiveness and nitrogen effects easy to demonstrate. BNF by all legumes is affected by management and mineral nitrogen, and the observations that are made in this demonstration are applicable to other legumes.

Treatments

The test legume used in this demonstration requires soil that is free of rhizobia. Refer to **Module 7** Demonstration 2 and *Methods in Legume-RhizobiumTechnology* for information on conducting a pot trial with soil.

Strain Selection. Select strains of rhizobia that differ in effectiveness (see **Module 4)**. NifTAL can supply effective strains for many species of legumes.

Management Treatments. Select a soil that is known to have low fertility or pH problems. Consult local soil scientists and extension agents about management strategies to increase yield in soils common to your region.

Follow the guidelines for maximum management treatments in **Module 7** Demonstration 2 or use local recommendations for the soil you are using for the demonstration.

In this Demonstration, as in the field trial (**Module 7** Demonstration 1), Maximal Management refers to management to maximize growth of the legume in the pot. Farmer management refers to no inputs other than the inoculation and nitrogen treatments used in this demonstration. Nitrogen refers to the addition of fertilizer nitrogen in amounts that will reduce BNF

Table D6/1-1. Treatments For Soybean

Inoculation	Management	Nitrogen
USDA 110	High	+
USDA 110	High	
USDA 110	Low	
Uninoculated	High	+
Uninoculated	High	
Uninoculated	Low	

Harvesting the Demonstration

Observations on plant growth and leaf color should be made and recorded. Plants should be cut at the soil level and the weight recorded if experimental results are of interest.

The root systems should be removed from the soil carefully so nodules are not detached. Observations should be made on the size and interior color of the nodules. Nodules should be removed, counted and weighed.

Record the data:

Treatment	Leaf color	Shoot weight	Nodule		
			Color	Number	Weight
USDA 110 High + N					
USDA 110 High - N					
USDA 110 Low - N					
Uninoculated High + N					
Uninoculated High - N					
Uninoculate Low - N					

MODULE 7: DEMONSTRATION 1

EFFECT OF FARM MANAGEMENT PRACTICES ON THE YIELD RESPONSE TO LEGUME INOCULATION

PURPOSE

- Demonstrate that inoculation can increase the yield of legumes.
- Demonstrate how farm management practices affect nodulation, nitrogen fixation, and the yield of inoculated legumes.
- Demonstrate that both good farm management and inoculation is required to maximize the yield of legumes.

CONCEPTS OF DEMONSTRATION

This exercise is useful to demonstrate that good farm management practices are necessary to obtain the full benefit from inoculating legume crops with rhizobia. Remember from the discussion in **Module 6** that plants require many elements and suitable conditions for growth such as phosphorus, potassium, water and suitable pH. If one of these necessary elements is available only in limited amounts, the legume will not grow as well as when there is a greater supply of the element. When legume growth is limited by low availability of necessary elements, the benefits the farmer obtains from inoculating his legumes with *Rhizobium* will be reduced. The legume does not require much nitrogen from BNF to grow if other elements are necessary for growth are missing.

This demonstration has a formal experimental design, replication, and defined controls and treatments. It is similar to standard trials that are conducted on experiment stations. The demonstration differs from many 'on farm' trials in its formal design and treatment definition.

FARMER RECOMMENDATIONS FROM RESULTS OF THIS DEMONSTRATION

Farmers should inoculate their legume crops to increase yield.

Farmers should use other inputs and good farm management to increase yield of their legumes in addition to using rhizobial inoculant.

Adding fertilizer nitrogen to the legume crop can reduce nodulation and BNF.

Management and inoculation can affect nodulation of the crop.

CONDUCTING THE DEMONSTRATION

Experimental Design. Six treatments demonstrate the concepts stated above. There are three basic treatments called Nitrogen Source:

1) **Inoculated (I)**. Plants inoculated with proper rhizobia. Nitrogen sources are BNF from inoculant and soil N.
2) **Uninoculated (U)**. Plants not inoculated. Nitrogen sources are soil N and BNF from native rhizobia, if present in the soil.
3) **Nitrogen (N)**. Plants not inoculated but supplied with fertilizer nitrogen. Nitrogen sources are fertilizer N and soil N.

Each of the three basic treatments will be compared at to levels of

Management:

1) **Maximal (High)** level management.
2) **Farmer (Low)** level management.

A **Split Plot** experimental design should be used. This design puts all three Nitrogen Source treatments for each level of management in one large plot (mainplot), and makes applying the Management treatments easier. The treatments should have four replications.

Therefore, the experiment has a total of 24 plots. Each of the four replications has two mainplots (Maximal and Farmer management), and each mainplot has three Nitrogen Source subplots (Inoculated, Uninoculated, Nitrogen), as shown in Figure D7/1-1.

Site selection. A good site would be a field that has not been planted with soybean or inoculated within the last ten years. Soybean is a good for demonstrating the benefits of inoculation and management since it requires a very special rhizobia that is usually not present in tropical soils. Other species can be used instead of soybean, but similar caution should be taken when considering the crop history of the site. A site where legume cultivation is not practiced would be best. This demonstration is also suited for field days at experiment stations.

Site characterization and treatment selection. Learn about the status of soil fertility and find out which management inputs increase legume yields at the site. It is useful to take soil samples at the site for analysis before deciding on management practices. The soil test will help to identify essential elements that must be applied to increase yield.

Based on soil test values and local knowledge, you can decide which inputs or management practices will increase yield of the demonstration legumes. Develop a management plan for the demonstration crops to ensure that the Maximum or High management treatments will have an increase in yield compared to no inputs or standard farmer practices.
Maximal management treatments can also be inputs other than adding soil amendments such as lime or fertilizer. The extension agent should also consider using pesticide, tillage,

or water management for the Maximal treatments if these practices are known to increase yield of legumes.

General fertility recommendations. It is best to rely on soil test values and local recommendations to choose the inputs for the Maximal management treatments. If those are not available, you can use the following general recommendations that will provide in excess of the legume's nutrient requirements for a wide range of soil types. pH: Lime acid soils to at least pH 5.5 and preferable to 6.0. Most acid tropical soils are highly buffered and the risk of over liming is slight. Use finely ground limestone and allow 4-6 weeks before planting.

K: Apply 150 kg potassium ha^{-1} prior to planting as KCl or K_2SO_4.

P: Use the following guidelines for phosphorus according to your soil type.*

kg P/ha	Soil Type
25-50	sands and sandy loams
100-200	light textured silt loams less weathered loam soils
100-200	highly weathered clay soils dominated by aluminum and iron oxides and hydroxides
200-400	volcanic ash soils

* Note the rates are on an elemental basis, not P_2O_5. Apply the P as single, double, or treble super phosphate.

Mg: Apply 50 kg Magnesium/ha as $MgSO_4.7H_2O$ (Epsom salts), or the Mg can be obtained from dolomitic limestone.

Zn: Apply 10 kg Zinc/ha. ZnSO4 is one common form but any zinc salt will be sufficient.

Mo: Apply Molybdenum at 0.5 kg Mo/ha using Na_2MoO_4.

S: You will not need sulfur if you used $MgSO_4$, K_2SO_4, or single super phosphate. If fertilizers without sulphur are used, apply $CaSO_4$ or K_2SO_4 to give 25 kg S/ha.

Micronutrients may not be required.

Broadcasting and incorporation of fertilizers should be uniform. It is usually easiest to weigh and broadcast fertilizers for each Maximal Management Mainplot. The one exception is Mo, since the quantity involved is very small. Mix Mo thoroughly with another material, or even better, mix with water and spray onto the field.

Plot layout. The attached drawings (**Figures D7/1-1, D7/1-2**) provide an example of this design. The field and plot layout can vary with conditions and species that is being planted. For convenience a typical plot size suitable for soybean, cowpea, or peanut has been

provided.

Randomization of the plots. This is a formal experimental design in addition to being a demonstration. Statistical analysis can be performed on the results. It is necessary that the treatments be assigned to the plots at random.

Write each main plot management treatment (Maximal, Farmer) on a piece of paper. Place the two pieces of paper in a container and select one. This treatment is assigned to the first main plot in the first Block (replication). The other treatment in assigned to the second main plot in the first Block. Repeat the process until all the main plots have been assigned a management treatment.

Now assign the three Nitrogen Source treatments to the three subplots in each mainplot by the same process. Each sub plot will have one of the Nitrogen Source treatments and **all three Nitrogen Source treatments will appear exactly once in each main plot.**

Planting and Management of the Demonstration. It is not possible to give specific planting and management directions for every legume at the many different sites extension agents may select. The following information may help you to design the demonstration. Information on the management of legumes at the demonstration site should be obtained from the local extension agents and farmers.

Seed and Planting density. Follow local recommendations and use good quality seed. Determine the viability of seed before you plant. A simple germination test in a container of soil will tell you whether seed quality may affect your demonstration. If germination is less than 85% but the seedlings are vigorous, increase the planting density to account for seed that will not germinate.

Determining the amount of seed for each plot. It is easiest to determine the amount of seed required for each plot by weight. For example, in this demonstration each plot has an area of 3m X 6m = 18m^2 or 0.0018 hectare (ha). If planting density is 400,000 plants per ha, then each plot requires 0.0018 ha X 400,000 seeds per ha = 720 seeds. Weigh a sample of 100 seeds to determine the **average** weight of a seed. For example, the weight of 100 soybean seed is often 15 g, and so the average weight of a seed is 0.15 g. In this case, each plot will require 720 seeds, weighing 0.15 g X 720 = 108 g.

Planting the field demonstration is more simple if the seed for each plot is weighed in advance and placed in a separate bag.

Source of inoculants. If possible, work with the professionals at the inoculant production facility in your region. If quality inoculant cannot be obtained locally, it can be requested for this demonstration from NifTAL, 1000 Holomua Road, Paia, HI 96779, USA. For those in SE Asia, write to BNF Resource Center, Rhizobium Building, Division of Soils, Department of Agriculture, Bangkok 10900, Thailand. There are other facilities that can supply inoculant and addresses can be obtained from NifTAL and BNFRC. When writing for inoculant indicate the legume species you are using in the demonstration.

Inoculation. It is very important that the seeds for the Uninoculated and Nitrogen treatments do not become contaminated with rhizobia from the inoculant. Seeds for each of the Uninoculated and Nitrogen plots should be weighed first, and put in individual bags and sealed so they are ready for planting. Do not get any inoculant on these seeds, or touch these seeds after handling the inoculant.

Seed for Inoculated plots should be uniformly inoculated with good quality inoculant. Follow the recommendations for inoculation in the handbook *Legume Inoculants and Their Use* and **Module 5**. Increase the rate of inoculant application if the inoculant is old or has been stored in conditions over 32◦C.

Seed for each plot can be weighed and put in plastic bags where they can be inoculated using the two-step method described in **Module 5**. The seed can also be inoculated in larger quantities and then weighed for each plot as described.

Planting. Inoculate as close to the time of planting as possible. Keep the seeds in a cooler or otherwise protect from heat when transporting to the field as mentioned in **Module 5**. To prevent contamination of the Uninoculated and Nitrogen treatments, it is usually best to plant and cover these treatments within a block before handling the inoculated seed. Separate workers can be assigned to a particular treatment. If the inoculant does not stick to the seeds well, it can be easily blown by the wind to the uninoculated plots, so it is important to handle the inoculated seeds carefully.
It is also important to have a well tilled seed bed at planting and to make sure that the soil makes good contact with the seed when the seed is buried. These factors help to provide uniform germination of the seed. Do not allow the inoculated seeds to lay exposed to the sun during planting. Cover the seeds in each plot immediately after planting, and irrigate the entire field as soon as possible.

Crop protection. Control insect and disease pests before severe damage occurs. Consult local entomologists and pathologists to determine the most likely problems to be encountered, and **develop a plan for recognition and control**. Know what pesticides will be required before the onset of the problem. Pest problems can begin as soon as the seed is placed in the ground. It is therefore important to think through the whole life cycle of the crop.

The Nitrogen Control Treatment. Fertilizer nitrogen is applied to uninoculated plants in the Nitrogen treatment plots. This Nitrogen treatment will provide information on the *yield potential* of the crop (how much the crop is capable of producing) when N is not limited at the two levels of management.

For a crop to meet its yield potential, you need to apply N frequently, and provide more than the crop needs. It is difficult to recommend an individual N application rate for each environment and species. The following recommended rate was calculated for fast-growing grain legumes.

We suggest the following approach to maximize yield potential of the Nitrogen treatments.

1. Apply N at the rate of 100 kg N/ha as a **sidedress** once every two to three weeks beginning at planting. Do not place the fertilizer in direct contact with the seed because the N may cause problems with germination.
2. Use urea or NH_3NO_3 as an N source. Do not use other N salts containing other nutrient.
3. Do not irrigate excessively because too much watering will cause leaching of N from the rooting zone.

If the Nitrogen treatment is conducted properly, there will be no nodules on the plants in the N plots, even in soils that have a native population of rhizobia that is compatible with the legume crop. The Nitrogen treatment plants will be getting nitrogen from the fertilizer, and not from BNF.

Early Harvest. You can use the early harvest to make visual observations of the nodulation and shoot growth in the different treatments. You can also collect data from the early harvest for statistical analyses. If you are only making visual observations you can simply harvest a few plants from each plot, group them by treatment, and record your observations on shoot growth and color, and nodulation. Use the information in **Table D7/1-1**, and other information in **Module 6**, **Figure 7-3** of **Module 7**, and *Inoculants and Their Use* to interpret your observations on nodulation and shoot color.

Final Harvest. Plots of grain legumes should be sampled at **harvest maturity**. This stage (R8) is well defined for some species such as soybean and bush bean, but may be more difficult to define for species such as peanut, which do not decline rapidly. We recommend you seek advice for those species. Your Nitrogen treatment plots may take longer to reach harvest maturity than the other treatments. In this case you may have to harvest the other treatments first, and delay harvest of the Nitrogen treatment plots until the plants reach the same stage of maturity.

The following are some suggestions for harvest which may be useful :

1. Use a long measured stick or other device to physically mark the harvest area.
2. When plants are cut near the soil surface, avoid getting soil on the plant sample since it can interfere with chemical analysis.
3. If your sample drying facilities are not sufficient to handle large quantities of materials, a subsample technique can produce quality data (low variance) and reduce the amount of labor required.
   a) Remove all plants from the harvest area. Weigh and record fresh (wet) weight of the plot sample.1.5
   b) Immediately subsample, at random, whole plants (15-20 are usually sufficient depending on variability within the plot and plant density).
   c) Immediately weigh and record the fresh weight of the subsample before there is any change in moisture of the plants.
   d) Dry subsample to constant weight at 65◦C and record dry weight.
   e) Separate seed from subsample and record the seed and stover weight.
   f) Total dry weight of the harvest area dry wt.of subsample

$$\frac{\text{dry wt.of subsample}}{\text{wet wt. of subsample}} * \text{wet wt. of harvest area}$$

   g) Seed yield of the harvest area =

$$\frac{\text{dry wt. seed in subsample}}{\text{dry wt. subsample (seed + stover}} * \text{dry wt harvest area}$$

This subsampling technique requires rapid handling of the wet subsample; a random sampling of plants from the whole plot; uniform moisture application within the plot; and careful attention that material in the subsample is not lost in handling.

4. If you are doing N analyses, grind the dry seed and stover separately, and save 10-15 g subsample of each for digestion. Protect the ground sample from moisture during storage since the nitrogen can be lost under moist, warm conditions.

Table D7/1-1. Explanation for situations found in inoculation trials.

Condition	Explanation
UNINOCULATED PLANTS	
1. No nodules on uninoculated control. Plants yellow.	No native rhizobia capable of infecting that legume.
2. Many small nodules scattered over root system. Plants yellow.	Native rhizobia are not effective at BNF with the host.
3. No nodules on uninoculated control. Plants deep green.	Soil high in mineral nitrogen. No native rhizobia compatible with that legume.
4. Small nodules on uninoculated control. Plants deep green.	Soil high in mineral nitrogen. Native rhizobia may be effective or ineffective.
5. Uninoculated control plants have many large nodules. Plants deep green.	Native rhizobia effective on that legume. Inoculation may not be necessary.
6. Plus nitrogen control plants nodulated. Nodules small, plants green.	Native rhizobia may be effective. Nodules not working because of fertilizer nitrogen.
INOCULATED PLANTS	
1. Inoculated plants have no nodules. Plants yellow or green.	Inoculation failure. Improper inoculant or rhizobia in the inoculant are dead.
2. Inoculated plants have small nodules and deep green color.	Soil high in mineral nitrogen. Nodules not working.
3. Inoculated plants have large nodules, red on inside. Plants deep green. Uninoculated plants yellow with small or no nodules.	Native rhizobia not effective. Inoculant rhizobia very effective.
4. Inoculated plants receiving soil amendments (phosphorus, potassium, etc.) larger, more vigorous than inoculated plants without amendments.	Need amendments for maximum BNF.

Source: *Legume Inoculants and Their Use,* p.34

Figure D7/1-1. Diagram of a typical experiment station inoculation trial by management level experiment. Experimental design is a split-plot design. Management level (Maximal, Farmer) are main-plots. There are three nitrogen source treatments (Inoculated = I; Uninoculated = U, Plus Nitrogen = N). This type of experiment demonstrates the interaction between inoculation and other management inputs.

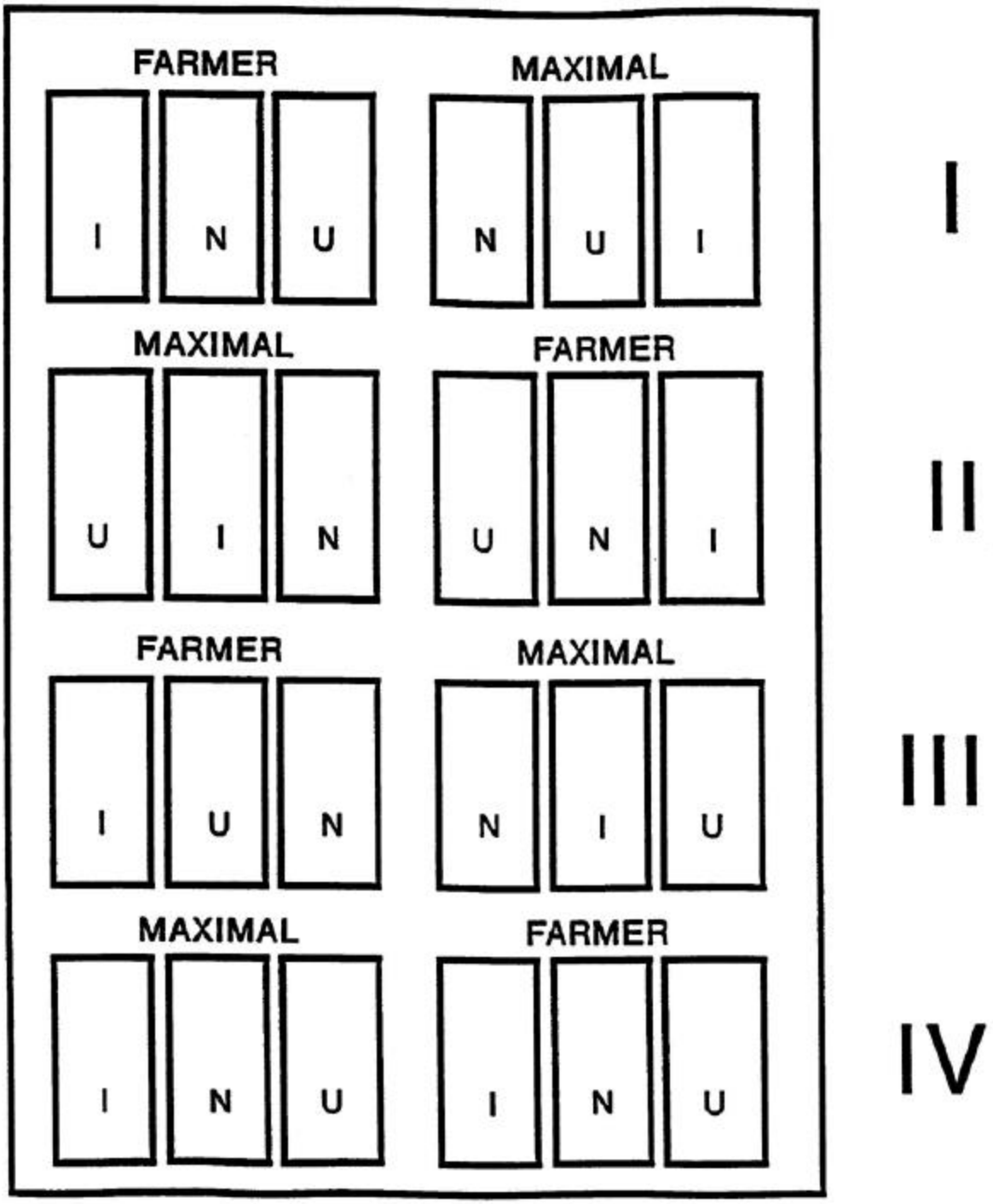
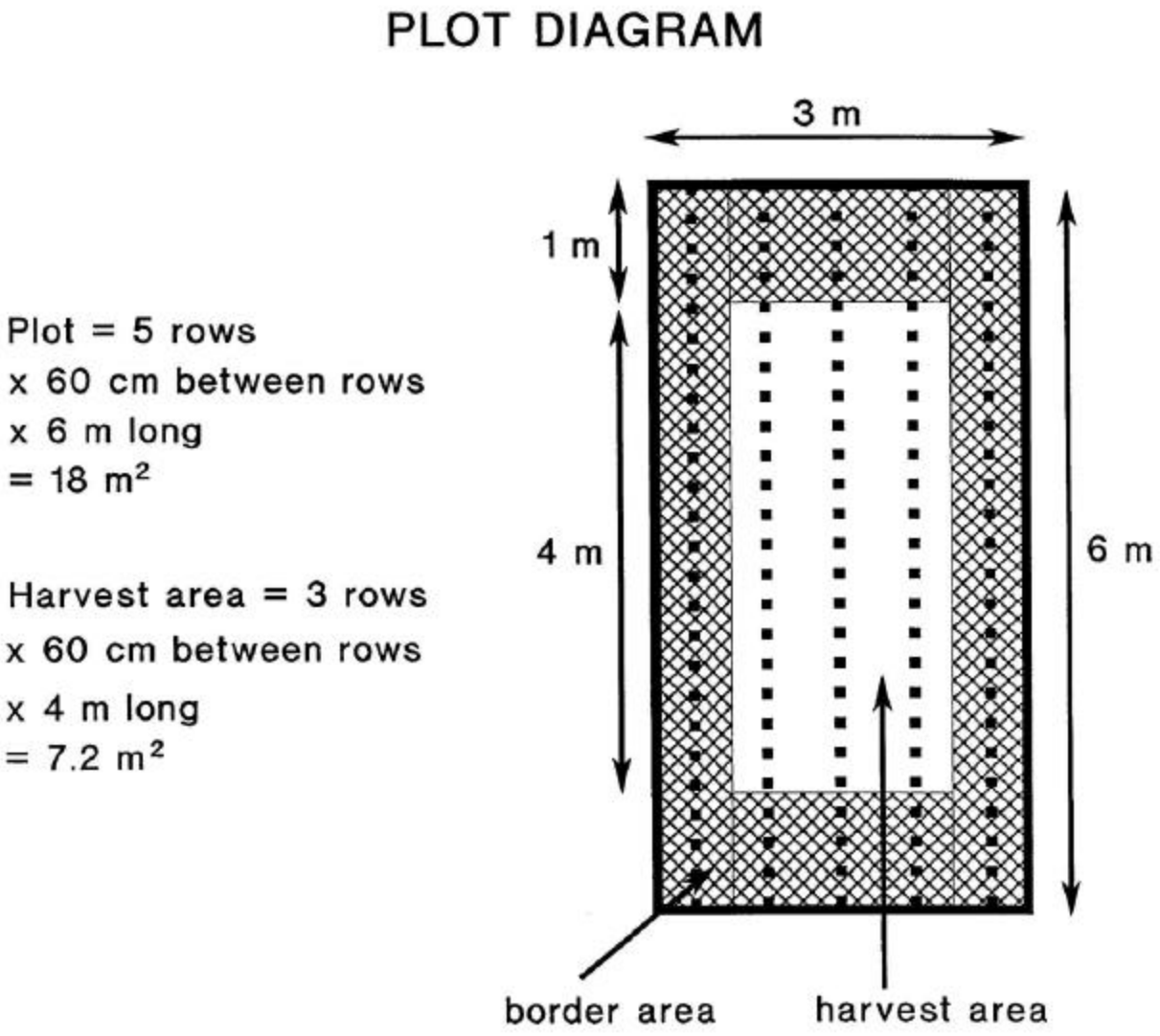


Figure D7/1-2. This diagram shows a typical plot in field experimentation. Border areas are not harvested. Border areas reduce the effect of treatments in adjacent plots.



MODULE 7: DEMONSTRATION 2

A POT EXPERIMENT TO DEMONSTRATE THE YIELD RESPONSE TO LEGUME INOCULATION

PURPOSE

- Demonstrate that rhizobial inoculation can increase the yield of legumes.
- Demonstrate the effect of different soils on the response to inoculation.

CONCEPTS OF THE DEMONSTRATION

This pot test is a quick and simple method to demonstrate that rhizobial inoculation can increase the yield of legumes. The advantage of the pot test is its simplicity. The extension agent can test the inoculation response of many different legumes on many different site soils without the extensive effort required by field trials. The design of the pot tests can also be easily adjusted for different purposes, such as looking at the effects of other inputs like fertilizers or lime on legume BNF, or for "grow out" tests of different inoculants. This demonstration will provide general instructions on how to conduct a practical pot test to measure the effects of BNF. It is up to the extension agent to adapt this information to use pot tests for solving specific problems at his site.

This pot test has a formal experimental design, with replication and defined controls and treatments. Besides using the pot test for demonstration, the extension agent can also record the results of the pot test for data analyses.

FARMER RECOMMENDATIONS FROM RESULTS OF THIS DEMONSTRATION

Farmers should inoculate their legume crops to increase yield.

Not all legume crops may benefit from inoculation in a certain soil.

CONDUCTING THE DEMONSTRATION

Caution : In pot tests measuring inoculation response, **you must be extremely careful to avoid contamination**. If your pots become contaminated with rhizobia from other soils, or if your uninoculated treatments are contaminated with inoculant, the results you get in the pot test will not be accurate.

Care should be taken that all utensils, pots, and implements are clean. Before starting any of the activities, it is a good idea to rinse all of the buckets, pots, screens, implements, etc. with a 10% bleach solution, then rinse them with fresh water. The implements can then be air dried on a clean tarp, and kept in clean plastic bags until you are ready to use them. If

you are comparing soils, you need to be especially careful to repeat the bleaching process between handling the different soils. These instructions will also review the special care required during inoculation of the seeds, watering, and maintaining the pots.

Keep soils used for pot tests cool! The native rhizobia in the soil will affect the response to inoculation. If the soils get too hot, the native rhizobia will die, and your results may not be accurate.

The Treatments. As in the field demonstration, we recommend three N source treatments:

Inoculated (I) with rhizobia

Uninoculated (U)

Nitrogen (N) fertilizer N, uninoculated

The pot test can be done either at farmer level fertility, with soil amendments, or both. The treatments can be modified, depending on the purpose of the test.

If the test is conducted in the greenhouse, the pots can be laid out in a completely randomized block design with four replications. There should be enough space between the pots to keep the plants from shading each other, especially if you are growing several legume species with different growth habits.

Soil Collection and Processing: Select the site soils using the same criteria as for selecting the field site in **Module 7** Demonstration 1. When collecting the soils, take a **composite** of samples from different locations in the field. Do not take the soil from only one spot.

1. Use clean utensils to collect soil from six locations within the proposed field site. Mine the soil to a depth of 20 cm after removing surface litter and the top 1.0 cm of soil.
2. If the soil is sufficiently dry, pass it through a 0.5 cm screen in the field. Otherwise remove the soil to a cool, shady place to air dry until it can be passed through the screen. Passing through a large mesh screen first will speed the drying process.
3. Proceed with the pot test as soon as possible.

Determining gravimetric moisture content to approximate field capacity moisture. The NifTAL manual (Somasegaran and Hoben) has a brief explanation (Appendix 21 p.346) of a quick method to determine percent soil moisture that approximates feld capacity. The moisture content of soil at field capacity is best for plant growth. Pots watered to field capacity will not drain. Water draining from pots can carry rhizobia and be a source of contamination. If the facilities to measure gravimetric moisture content are not available, the pots can just be watered carefully until a point just before drainage occurs. Care should be taken that draining water does not move toward any other pots.

To determine gravimetric moisture:

1. Select a 1000 - 2000 ml plastic cylinder or metal can and drill a hole at the bottom. The hole allows air to escape when water is added to the cylinder.
2. Take a random subsample of screened air-dried soil and fill the cylinder. Tamp the cylinder to a similar consistency as used in the pots.
3. Cover the surface of the soil with a paper towel or filter paper disc and pour a small quantity of water (100 ml) slowly onto the surface. Try to obtain an even movement of the water through the column.
4. Cover the vessel to avoid evaporation and wait 24 hours. The water **should not** reach the bottom of the cylinder.
5. After 24 h equilibration period there should be a sharp line where the water stopped moving in the soil column. Collect a sample of soil for moisture determination from about 5 cm above the wetting front.
6. Place the wet soil in a weighed dish (record weight of dish), weigh and record the weight of the wet soil plus dish. Dry the soil at 100◦C until it reaches a constant weight. Weigh oven-dry soil and dish.
7. The gravimetric moisture fraction on an oven-dried basis is calculated by:

$$\text{gravimetric moisture fraction} = \frac{\text{wet weight - dry weight}}{\text{dry weight - dish weight}}$$

where:

Wet weight = weight of wet soil plus dish weight

Dry weight = weight of oven dry soil plus dish weight

Dish weight = weight of drying dish

Determining the amount of oven-dry soil per pot. It is important to know the equivalent amount of oven-dry soil per pot if the soil will be amended with fertilizers. The amounts of fertilizers to use are calculated on an oven-dry weight basis. After the soil collected from the experimental field has been air-dried, screened and thoroughly mixed, a subsample of soil should be taken to determine air-dry moisture content. The moisture fraction is used to calculate the equivalent amount of oven-dried soil in each pot.

1. Bulk together and mix the soil that will be used to fill the pots. Take at least 15 subsamples (10-20 g each) of soil and mix. Cover the air-dried bulk soil and store in the shade so that the moisture status does not change.
2. From the mixture of subsamples in step 1, take three subsamples and place each in a weighed dish (record dish weight) and record air-dry weight plus dish weight.

Place the samples in the oven at 100◦C. Determine the Air-Dry Moisture Fraction as in step 6 above.

3. Weigh some empty pots to determine pot weight and variability. Clay pots will usually require individual weighing whereas plastic or other manufactured materials will be sufficiently uniform to use an average weight for the pots.
4. Add air-dried screened soil to a 7-8 liter pot until soil is within 2-4 cm of the top. Drainage holes may have to be sealed with tape to prevent loss of soil. Determine the net weight of air dry soil in each pot by calculating (the weight of air-dried soil and pot) - (the weight of pot).
5. Calculate the equivalent amount of oven-dried soil in the pot by :

$$\text{Oven-dried soil (g)} = \frac{\text{air dried soil (g)}}{(1 + \text{air-dry moisture fraction})}$$

For example, if the air-dried soil was found to have an Air-Dry Moisture Fraction of 0.12 on an oven-dry weight basis, and the net weight of air-dry soil added per pot was 7.84 kg, then the equivalent amount of oven-dried soil would be:

$$\text{Equivalent wt. of Oven Dry Soil} = \frac{7.84 \text{ kg air dry soil}}{(1 + 0.12)}$$

$$= 7.00 \text{ kg}$$

Adjusting the pH. The soil pH should be adjusted to about 6.0 to avoid problems with micronutrient availability. The amount of amendments added to the soil can be approximated based on local experience and practices. If pH meters or soil testing kits are available, we recommend making a liming curve to calculate the amount of lime needed to correct acid soil conditions (pH less than 6.0). For rapid equilibration with the soil, the best material to use is $Ca(OH)_2$, and not $CaCO_3$. There are many ways to determine the lime required to bring the pH to 6.0. Titration of a 1:5 (soil-water) slurry with Ca(OH)2 is common (see Somasegaran and Hoben, NifTAL training manual, 1985; Appendix 16, p.328).

1. Take subsamples totaling about a kilo of your soil and mix as for determining the soil moisture. Known amounts of $Ca(OH)_2$ can be weighed out and added as a dry ingredient to a known amount of **air-dry** soil (0, 25, 50, 100, 200, 400 mg $Ca(OH)_2$

per 100 g soil), in duplicates. Add 100 ml water and stir vigorously to make a paste. Cover and let stand with periodic stirring for 3-4 days (90% of the reaction will be complete by that time) and take the pH. After equilibration, add 400 ml deionized water, stir, and take pH after 30 min.

2. Make a curve that plots mg of liming material per kg soil to resulting pH. **Do not oven dry the soil samples to be used for the liming curve**.
3. Based on this curve, select a liming rate by converting the amount of liming material required to reach a pH of 6.0, to the amount of lime needed for the soil in the pots. You will need only 80% as much $Ca(OH)_2$ as $CaCO_3$.
4. Apply the $Ca(OH)_2$ or $CaCO_3$ dry and thoroughly mix with the air-dried soil. Mix the soil and liming material in a clean cement mixer, or on a clean tarp. The soil does not need to be weighed for this process. Instead, use approximations based on volume. For example, you can calculate the weight of the soil in ten pots, and add the appropriate amount of liming material to mix with the soil. Other soil amendments such as fertilizers can be added at this time.
5. After the soil has been added to the pots, it should be watered to field capacity (see following). Planting should be delayed for 3-4 days if $Ca(OH)_2$ was used, and for 10-18 days if $CaCO_3$ was used to lime the soil. This delay will allow the lime to equilibrate. Again, keep the pots in the shade and do not let them overheat in the sun.

Other Amendments. The pot test can either be conducted at farmer level fertility or with added amendments. There are advantages for both practices. Conducting the experiment at farmer level fertility will give a more accurate assessment of the response to inoculation under farm conditions. Using amendments which can improve the growth of the legume will demonstrate the **potential** for increasing crop yields with BNF.

Phosphorus is one of the most important elements which may be limiting in tropical soils. It can be provided as potassium phosphate, mono or triple superphosphate. Do not use the ammonium phosphate fertilizers as these will add nitrogen to your system. K can be supplied as potassium phosphate or potassium sulfate. If you use dolomite to lime your soil, you will have added adequate Mg. Otherwise Mg is available as Magnesium sulfate (Epsom salts). Sulphur is present in single superphosphate, magnesium sulfate, or can be added as gypsum (calcium sulfate).

General recommendations for providing major elements which may improve crop growth are:

		mg Element per kg soil (oven dried)
Phosphorus	(P)	75
Potassium	(K)	75
Magnesium	(Mg)	20
Sulphur	(S)	20

To calculate the amount of fertilizer you will need to provide the recommended amount of the individual elements, you first need to know the percent of the element in the fertilizer. This information is usually listed on the fertilizer bag, and may vary with manufacturers. For example, to provide 75 mg P per kg soil, from triple superphosphate (commonly about 20% P) use the following:

$$\frac{\text{75 mg required}}{\text{20 P in fertilizer}} = \text{375 mg fertilizer per kg soil}$$

You need 0.375 gram of triple superphosphate per kg of oven dried soil. If you know from your earlier calculations that each pot will hold the equivalent of 7.00 kg of oven dried soil, you will need 2.63 g of triplesuperphosphate per pot.

If pure salts are used calculate the proportion of each element in the compound. The proportion of each element is the atomic weight of the element (times the number of atoms of the element in the molecule) divided by the molecular weight of the molecule. Soluble fertilizers can be added to the pots as solutions (see section "Watering to field capacity," or mix the dry fertilizer to the air-dried soil at the same time as adding the lime.

Micronutrients are usually not a problem if the pH of the soil is properly adjusted. If you suspect that you may need to add micronutrients, see a soil fertility specialist for his recommendations.

Nitrogen Treatment. The nitrogen treatment pots should receive enough N to inhibit nodulation of species that have native rhizobia in the test soil. There are large differences between species in their ability to accumulate nitrogen during early growth. Soybean, for example, can accumulate up to 300-500 mg N/ plant after 30-35 days of growth, compared to slower growing *Leucaena* which accumulates only 30-40 mg after 50 days growth.

Applying 50 mg N per kg soil (oven dry equivalent) three during the pot test should be sufficient to inhibit nodulation of vigorously growing grain legumes, as long as the N is not leached from the pot or denitrified. **This application rate should be adjusted downwards for legumes which accumulate less N, such as forages, or under conditions where high temperatures may cause toxicity**.

Use urea or ammonium nitrate (NH_4NO_3) as the fertilizer N source since other N fertilizers will also add other nutrients. Use the same calculations as in the previous section to determine how much N fertilizer will be required for each pot. The N can be added in a liquid form, as described earlier, and washed into the soil with water to disperse the salts.

Apply 50 mg N per kg soil (oven dry equivalent) after seed emergence as high N levels may affect germination.

Watering to Field Capacity. To follow the instructions in this section, you need to have determined gravimetric moisture content and the oven dry weight equivalent of the soil in your pots as described in the earlier sections. Watering to field capacity is done by weighing the pots. The total weight of the pot consists of the weight of the pot itself, the weight of the soil (oven-dry equivalent) and the weight of moisture in the soil at field capacity.

Clay pots usually have to be individually weighed due to pot weight variation. Manufactured plastic pots are sufficiently uniform that a single weight may be used to calculate total weight.

For example :

Weight of pot	=	0.25 kg	
Soil (oven-dry equivalent)	=	7.00	
Water at field capacity	=	2.24	(0.32 Moisture Fraction at Field Capacity)
Total	=	9.01 kg	

Gravel or other dry mulch on the surface of the soil in the pots may help to prevent cross contamination between treatments. The gravel mulch dries out quickly between watering and rhizobia do not survive well on the mulch. If a dry mulch barrier is used, its weight should be added to the total weight of each pot.

1. Add water (including amendments) to air-dry soil in pot until the total desired weight is achieved.
2. Keep pots at field capacity after planting by weighing and adding water to make up losses. If this procedure is followed properly, no water should drain from the pots during water additions.

If you do not have the facilities to determine field capacity or to weigh the pots, you can

estimate field capacity by slowly adding a measured amount of water to a test pot until the water just starts to drain. You can then use a slightly smaller volume of water to wet the rest of the pots. To maintain the moisture in the pots when the legume is growing, you will need to add the water slowly to avoid draining, since the draining water is a source of contamination.

Inoculation. Inoculate seeds with the correct rhizobia using the two-step method described in **Module 5**. Use care to avoid contaminating the uninoculated treatment seeds.

Planting. Use care to avoid exposing the inoculated seed to heat or direct sunlight. Keeping the seeds cool will insure the best survival of the rhizobia. Use clean utensils to plant the uninoculated treatments first and cover before handling the inoculated seeds.

1. Plant 8 to 10 seeds/pot for large seeded species (soybean), 10-15 seeds/pot for moderate seed size species, and 20-40 seeds/pot for small seeded species. Planting seed hilum down (large seeded species) often results in better emergence uniformity. Cover the seeds and add a small amount of water to each pot to be sure the seed has good contact with moist soil. Add gravel mulch if desired.
2. Select uniform seedlings, and thin plants 8-14 days after emergence. Large seeded species with early vigor (like cowpea) will be thinned earlier than slower growing species (like leucaena) or small seeded species. Thin large seeded varieties to two to three plants per pot depending on time of year. (Seasons with greater solar radiation reduce the need for greater plant number). Smaller seeded and slower growing species can be thinned to 6-15 per pot depending on species. Thin to the same number of plants per pot for all treatments.

Harvest. Fast growing species such as cowpea and soybean can usually be harvested in 33-45 days, depending upon growth rate at individual locations. You should be able to see responses to inoculation 21-27 days from emergence. Slower growing species will require a longer growth period. The best time for harvest will vary, depending upon conditions. It is best to maximize and sustain early growth. Harvesting too early can mean that real differences have not yet appeared. If the pot experiments are maintained beyond the system's capability to sustain rapid growth, real treatment differences may disappear.

It is useful to make visual comparisons between plants. Even though treatments may appear to be the same size, they may actually have large differences in total N. Slight color differences usually mean large differences in the % N in the shoot. The +N control for a species can be used as a standard to compare growth and color differences.

A useful method for making visual comparisons is to compare the size and color of recently expanded leaves, instead of looking at the whole plant. Compare leaves or *trifoliolates that are the same number of nodes from the base of the plant. If the recent growth rate has been affected by inoculation, differences in leaf area between treatments should increase toward the newer growth at the shoot apex. This approach will help to track treatment

differences during growth.

Ideally, harvest should be undertaken when treatment differences are greatest. If there are no compatible native rhizobia in the soil, the uninoculated plants should remain yellow. In this case, if the uninoculated plants begin to turn green (usually greening first takes place in interveinal portions of newer leaves), there may be contaminants on Uninoculated plants. Harvest should not be delayed too long after this point or real treatment differences may begin to disappear.

1. Plants should be cut at the soil surface, shoots dried at 60-70◦C until constant weight and then weighed. The shoots can be ground for digestion if total nitrogen will be determined.
2. Recover nodules to determine treatment effects on nodule number and dry weight. Carefully wash roots free of soil and remove nodules. Dry these at 60-70◦C and weigh.

Use **Table D7/1-1** to interpret your observations of nodulation and plant growth.

MODULE 8: DEMONSTRATION 1

TAKING CONTROL OF THE TECHNOLOGY TRANSFER PROCESS

PURPOSE:

- Motivate participants to accept the challenge of BNF technology transfer.
- Identify the recipient of BNF technology transfer.

CONCEPT OF THE DEMONSTRATION

Successful delivery of this training course requires instilling in participants their importance in the technology transfer process. Unless participants realize this key role, farmers will receive no benefit. Each participant must also be able to recognize the real needs, circumstances, and life style of the farmer/recipient. This knowledge will help extension workers to develop appropriate plans and strategies for getting the technology into the hands of farmers.

CONDUCTING THE DEMONSTRATION

A: Motivation

1. Spend some time at the beginning of this module encouraging the participants and praising them for their commitment to learning about BNF technology. The instructor's major task is communicating his or her respect for the participants and their new role. Building rapport with participants will encourage them to participate in later exercises.
2. Have participants test themselves on the attitude questionnaire on page 8-2. After about 5 minutes, ask them for feedback about their experience in the process. This test will help participants become aware of their ability to perform technology transfer tasks. There are no wrong answers in this type of test. Many "no" answers simply implies the need to assign the task of teaching technology transfer to another person whose personality and attitudes are more appropriate to the task.

B: Identifying the technology recipient

1. This can be a pleasant and exciting activity. The goal is to make a list of qualities one could expect in the farmer who would be using inoculants. The group makes a list of typical characteristics (i.e., demographics, farm size, crops, religion, etc.). Although there are several ways to conduct this exercise, one way to begin is to be

prepared with a large sheet of paper on which you have drawn the figure of a person. As ideas are offered, write these inside your drawing. This symbolic representation should be displayed during the entire presentation of **Module 8**. A continuing focus should be the realities of the technology recipient's life, interests, and needs.

2. If there is time, real in-country case studies can be reviewed considering the representative farmer that has been envisioned. Changes may be expected and perfecting the representation can only improve the participants' chances of success in the field.

MODULE 8: DEMONSTRATION 2

COMMUNICATION SKILLS PRACTICE

PURPOSE

- Identify the goals of and blocks to successful communication.

CONCEPT OF THE DEMONSTRATION

Effective teaching is done by people who know that facilitating learning is their main task. Reaching adult learners is different than teaching children. Participants can gain confidence through participation and exposure to certain truths. These truths have been gained through actual experience in teaching and research made into the effects of teaching methods and styles.

CONDUCTING THE DEMONSTRATION

Several slides and handouts center around this section. While working through the module these two demonstrations will give participants an experiential learning lesson.

A: The Secret. This simple exercise teaches a powerful lesson and is usually very amusing. The lesson proves the difficulty of communicating orally because the probability of distortion and reinterpretation of the message is great.

1. Simply decide on a short message (10 to 12 words written down) and have participants whisper it to each other around the room. Expect to find the message changed in an often humorous way.

B: Abstract Forms. This exercise teaches another lesson on the difficulties of verbal communication.

1. Reproduce one of the sets of abstract figures in the handout section or draw one of your own. These should be made on thick paper or cards (5" X 8" is a good size) that can not be seen through. Make enough copies for half of the participants.
2. Form the participants into pairs. Distribute the cards to one person in each pair and caution them not to let their partner see the design. Pass out blank cards to the partners.
3. Persons holding design cards must verbally communicate ONLY instructions on reproducing the design. No hand motions or other communication methods may be used - **strictly verbal instructions only**. Give each pair 5-10 minutes for giving instructions and drawing. This is quite difficult and

some frustration may be expected, although it is also humorous to most people.*

4. Let pairs examine the two cards and discuss the process for 3 or 4 minutes. Then ask for volunteers to report on their experience and what they learned. This process should reveal an increased respect for the challenges of communicating verbally and the need to use more than one method of teaching. Point out the distinct advantages of using all three teaching methods, i.e., Show it, Tell it, Do it.

*Some consideration should be given to cultural appropriateness when planning this exercise.

MODULE 8: DEMONSTRATION 3

PLANNING A COMPREHENSIVE BNF TRANSFER PROGRAM

PURPOSE

- Practice planning and using a systematic approach to technology transfer.
- Complete a national, regional, and local BNF technology transfer plan.

CONCEPT OF THE DEMONSTRATION

It is important to convey to participants the advantages of planning. This demon-stration conveys this message and results in a plan that may be used as a follow-up to the course.

CONDUCTING THE DEMONSTRATION

Give participants a chance to practice planning. The instructor should consider carefully how to divide participants into groups. Decide whether to divide participants into all three groups (national, regional or local levels) or to concentrate on one or two levels, and consider whether the participants will be able to put their plan into action. Frustration over not being able to implement any of their plan might abort the participant's attempt to transfer BNF technology. If participants understand the potential power of presenting a well thought out plan to their supervisor, they will be enthusiastic about this process.

1. Following the presentation of the planning material in **Module 8**, participants should be divided into groups. Even if it has been decided to cover fewer than three planning levels, small groups (five-seven persons) should be formed. It is usually good to let people form themselves into groups because by this time in the course, relationships may have been formed. Several groups can cover the same level. The advantage of this approach is an overall improvement in the final plan as each group presents a different perspective or level of experience.
2. Give the groups at least 1 hour to form their plans overnight is even better. Pass out large sheets of paper and ask participants to follow the modules instructions for planning. A quick review of the planning slide would be helpful.
3. Reportage should be rewarded by a formal presentation of groups and applause. Displaying the plans as they are presented will encourage discussion of differences and similarities, agreement or conflict, and comprehensiveness of the plans. If possible, someone should be transcribing the presentations as they are given. The optimum goal of this exercise is to produce a three-level plan for the participants to take home as an output of the course.

GLOSSARY

Anabaena azollae -This relationship is useful in rice-based crop systems throughout Asia.

Azolla-Anabaena **symbiosis** -A biological nitrogen fixation relationship between the aquatic fern Azolla and the cyanobacterium Anabaena azollae. This relationship is useful in rice-based crop systems throughout Asia.

Aeration -Supplying or charging liquid with a gas to be used in respiration.

Ammonia -A colorless gas produced in the manufacture of fertilizers and found in a wide variety of nitrogen containing organic and inorganic chemicals. In developing nodules, ammonia is needed for attachment to a compound provided by the host, forming an amino acid.

Ammonium (NH_4) -A chemical ion that is produced during BNF.

Bacteroids -Pleomorphic forms of rhizobial cells found in the nodules.

Biological Nitrogen Fixation (BNF) -The conversion by certain algae and soil bacteria of atmospheric nitrogen into organic nitrogenous compounds assimilable by plants.

Blocks -recommended division of test areas to ensure similarities in test conditions.

Break-even analysis -the level where increased income due to inoculation equals the cost of inoculant.

Caesalpinoideae -A subfamily of Leguminosae, with irregular flowers. One of the poorest nodulating subfamilies of Leguminosae.

Carpel -The central ovule-bearing female organ of a flower consisting of a modified leaf forming one or more sections of the pistil.

Competitive -Those strains of rhizobia that are faster at forming nodules than other strains.

Cover crops -A temporary crop, such as rye or clover,planted to protect the soil from erosion in winter and to provide humus or nitrogen when plowed under in the spring.

Cross inoculation group -A collection of legume species that will develop nodules when inoculated with the rhizobia obtained from the nodules from any member of that legume group.

Cycle -The completion of a series of events making a full circle.

Denitrification -When nitrate is changed back into nitrogen gas(N_2), permitting its return to the atmosphere. This is carried out by bacteria found in soil and water.

Dicotyledonous plants -One of the two major divisions of angiosperms, characterized by a pair of embryonic seed leaves that appear at germination.

Dusting method -The least effective method of seed inoculation and not recommended. Powdered inoculant is mixed with dry seed resulting in poor adhesion.

Effective -When the rhizobia and legumes are well matched and nodules form that will fix nitrogen.

Enzyme -Any of numerous proteins or conjugated proteins produced by living organisms and functioning as biochemical catalysts in living organisms.

Fertilizer use efficiency -The fraction of nitrogen applied that is actually taken up by the crop.

Flagella -Thread-like structures that make rhizobia motile.

Forage legumes -Legumes grown in pastures for animal feed.

Fungicides -Seeds are often coated with these chemicals for fungal control. Fungicides are usually harmful to rhizobia. Soil inoculation is recommended when they are used.

Grain -Cereal grasses or the small hard seeds or fruit from cereal grasses.

Green manures -A growing crop, especially a legume, that is plowed under the soil to improve fertility.

Grow out test -A method of testing the nodulation ability of an inoculant. Seeds of host legumes are inoculated and checked for nodulation after three to four weeks of growth.

Harvest index -The weight of grain or other economic yield divided by the weight of shoot and grain. Used to evaluate the benefit of legumes to the nitrogen fertility of soil.

Ineffective -When the rhizobia and legumes are not well matched and even though nodules may form, they will not fix nitrogen.

Infection process -The series of events whereby a rhizobia enters the root cells of a legume.

Infection tunnel (infection thread) -The passageway by which the bacteria moves through several root celllayers of the plant to the site where the nodule will develop.

Inoculant -The carrier material used to introduce rhizobia to leguminous seeds. The ratio of inoculum to carrier is 1:1 to 1:2, depending on the absorption ability of the carrier.

Inoculation -In Rhizobium technology, infecting soil or legume seeds with rhizobia.

Inoculum carrier -A highly absorbent non-toxic material used to mix with inoculum. Peat, finely ground or granular In texture, is the carrier most commonly used.

Inoculum -A broth culture of rhizobia used to make inoculant.

Inorganic N -Nitrogen derived from mineralization, e.g., N in the form of NO_3 and NH_4.

Insecticides and Herbicides -These chemicals are often applied in granular form to the furrow. They are only harmful to rhizobia when applied to the seeds directly.

Intercrops -The secondary crops growing between the rows of a principal crop.

Introduced rhizobia -The rhizobia put in the fields through farmer's inoculants.

Kwashiokor -Severe malnutrition occuring especially in children, characterized by anemia, edema, potbelly, depigmentation of the skin, and loss of hair or change in hair color.

Law of the Minimum -Yield in a farmer's field is limited by a single factor; only when that factor is added to the crop will yield increase.

Legume-rhizobia symbiosis -Intimate association of rhizobial bacteria and leguminous plants that leads to Biological Nitrogen Fixation (BNF).

Legumes -Any plant of the family Leguminosae, characteristically bearing pods that split into two valves with the seeds attached to the lower edge of one of the valves.

Limiting nutrients -The nutrient in the smallest supply determines the size of the farmer's yield. This nutrient is called the limiting nutrient since the amount of this nutrient determines the yield of the crop.

Marginal analysis -the calculation of increased income, above the cost of inoculation, due to investment in the inoculant.

Mimosoideae -A subfamily of Leguminosae with flowers collected into a dense head. The subfamily with the second highest incidence of nodulation.

Native rhizobia -Rhizobia that are already living in the soil.

Nitrogen mineralization -The conversion of soil organic N to inorganic forms of N.

Nitrogen gas (N_2) -The inert form of nitrogen found in the atmosphere which is converted to ammonium by BNF or by chemical fixation.

Nitrogen harvest index -A measure of the efficiency of recovery(harvest) of the total nitrogen in a crop.

Nitrogenase -An enzyme which enables rhizobia to convert N2 to NH3(ammonia).

Nodules -A small, knoblike outgrowth, such as those found on the roots of most leguminous plants.

Non-parametric statistics -Appropriate statistical analysis for a series of on-farm inoculation trials.

On-farm research -A logical sequence for developing farmer recommendations to inoculate legumes and assess the benefit farmers derive from inoculation.

Organic N -Nitrogen derived from dead and living organisms, e.g., N in the form of amino acids or proteins.

Papilionoideae -A sub family of Leguminosae with characteristic 'butter-fly' shaped flowers. The sub family with the highest incidence of nodulation.

Persistence -Referring to the survival of introduced rhizobia.

Photosynthesis -The process by which cells in green plants convert light to chemical energy and organic compounds from inorganic compounds, especially carbohydrates from carbon dioxide and water, and release oxygen at the same time.

Plant nutrient -The essential elements required by a plant for growth.

Plant infection tests -A method of estimating the number of rhizobia in inoculant or soil samples. A serial dilution is made of the sample and an aliquod of each dilution is added to a host plant. The resulting nodulation or absence of nodulation will indicate presence of rhizobia.

Promiscuous -A term used to describe the legume that can form symbiotic associations with rhizobia from many other hosts.

Range plants -Pasture legumes or other plants growing naturally in fields.

Recommendation domains -groups of farmers that have similar crop systems, management, climate, and soil. Farmers within a recommendation domain can expect to benefit similarly from inoculation.

Residual Nitrogen -The nitrogen that is left in the soil after a crop has been harvested and decomposition of soil organic matter has taken place. This residual nitrogen is then of benefit to the next crop.

Rhizobia culture -Growing rhizobia in a nutrient medium under artificial conditions.

Rhizosphere -The region around and close to the root.

Root hair -A thin hairlike outgrowth of a plant root, that absorbs water and minerals from the soil. It is on the root hair that rhizobia will enter the root.

Rotational crops -Changing crops from year to year to resupply the soil with nutrients that have been depleted.

Saprophytes -Organisms which live on the organic matter in the soil.

Seed Pelleting -Inoculated seeds are coated with a layer of powdered lime or phosphate. The pelleting material forms a hard coating around the inoculant as protection from adverse weather conditions, protection against soil additives, insects, soil acidity, etc.

Senescence -Aging and decaying, as in legume nodules.

Slurry inoculation -A seed inoculation method which requires a slurry made by mixing sticker with inoculant. This slurry is then coated on the seed.

Soil organic matter -Plant and animal residue that gradually decompose, releasing nutrients.

Starter Nitrogen -A small amount of nitrogen farmer's apply to their legume crop at planting.

Stover -The dried stalks and leaves of a cereal crop that remains after the grain has been harvested.

Strains -Rhizobia of the same species which are genetically distinct.

Swartzioideae -A small subfamily of Leguminosae that is relatively unimportant economically with nodulation not well known.

Two-step inoculation -A seed inoculation method in which seeds are first uniformly wetted with a sticker. Inoculant is then added and coated on the sticky seeds.

Vascular tissue -The connections that enable the host to feed sugars from photosynthesis to the rhizobia and the rhizobia to transfer fixed N_2 (ammonia) in the nodule to the plant.

Wilcoxon's Signed Rank test for paired data –A non-parametric statistical test useful in inoculation trials since inoculated and uninoculated treatments are paired on each farm.

Yeast mannitol agar –a solidified culture media of yeast sugar alcohol and mineral salts used in the culture of rhizobia in the laboratory

SLIDE NOTES AND EXPLANATIONS

1. M1/1 The Nitrogen Cycle. Gaseous nitrogen in the air is converted into a biologically useful form through biological nitrogen fixation in legumes and through chemical fixation in the fertilizer manufacture process.

2. M1/2 The Detailed Nitrogen Cycle. Crops require more nitrogen than any other plant nutrient. Nitrogen transformations in the biosphere are controlled by bacteria; nitrifying bacteria convert nitrogen in organic matter to ammonia and then nitrate; denitrifers convert nitrate in the soil back to atmospheric nitrogen; rhizobia convert this nitrogen back into ammonia within the root-nodule.

3. M1/3 The Leguminosae. The Leguminosae is the third largest plant family, with over 20,000 species represented in the temperate and tropical habitats, from herbs to large trees. Some of the worlds most important high protein foods are legumes, such as beans, soybeans, peas, peanuts and alfalfa.

4. M1/4 Rhizobia are Soil Bacteria. Rhizobia are rod-shaped soil bacteria which can be either free living or symbionts. When the proper legume root arrives in their soil habitat they can invade the root and eventually come to reside inside the host structure called a root-nodule. From inside the nodule, they carry out the process of biological nitrogen fixation.

5. M1/5 High Protein Products from the Symbiosis. The high nitrogen levels in a well nodulated legume give rise to high protein levels in the harvested plant. Soy sauce, tofu, and peanut butter are examples. Legumes such as peanut and soybean also produce high quality oils used in cooking.

6. M1/6 Matching the Plant and the Microbe. This field demonstrates two important points: 1) inoculation is necessary for proper growth of the plant (peanut, in this case) when the rhizobia are not present in the soil, and 2) different genetic varieties of the host require different rhizobia for an effective symbiosis.

7. M2/1 Subfamily Caesalpinoideae. A flower of *Bauhinia* sp. shows floral morphology typical of the species in the subfamily Caesalpinoideae.

8. M2/2 Subfamily Mimosoideae. Inflorescence of *Acacia farnesiana* which consists of small florets arranged to give a "head" common to species in the subfamily Mimosoideae.

9. M2/3 Subfamily Papilionoideae. "Butterfly" flowers of the subfamily Papilionoideae are typified by *Lathyrus* sp.

10. M3/1 What Rhizobia Look Like. These are rod-shaped bacteroides of *Bradyrhizobium japonicum* stained with fluorescent antibodies.

11. M3/2 Effective Symbiosis Nodule Color. Sections through effective nodules show the presence of leghemoglobin. Note the red color similar to human blood.

12. M4/1 The Infection Thread. Rhizobia enter the legume host usually through penetrating a root hair. The invagination of the host cell results in an "infection thread," by which the rhizobia travel to the site of the nodule primordia.

13. M4/2 Nodulated Soybean Root System. This soybean root system is covered with root-nodules. Within these structures are millions of rhizobia. It is within these nodules that nitrogen fixation occurs. The host expends a lot of energy maintaining these active nodules in return receiving ammonia which is converted to amino acids and proteins.

14. M4/3 Nodulated Peanut Root System. Nodule shape is determined by the host legume. Note the many smooth spherical nodules.

15. M4/4 Nodulated Birdsfoot Trefoil Root System.

16. M4/5 The Inoculated Seed. Farmers use the rhizobia by coating seed prior to planting with peat which carries the bacteria. Peat-based inoculants are available commercially to farmers in developed countries and increasingly in developing countries.

17. M4/6 Ineffective Native Rhizobia. The small plant on the left is a poorly nodulated alfalfa grown in a Washington state field. The native soil rhizobia were parasitic on alfalfa, and inoculation (in spite of severe competition) benefited the plants, as shown on the right.

18. M4/7 Inoculation Response in Soybean. This sandy soil in Florida showed a dramatic response to inoculation with soybean inoculant, as shown by

the rows on the left. The two rows on the right are uninoculated plants. The economic return in such a situation is quite high.

19. M4/8 Inoculation Response in Alfalfa. Perennial legumes such as alfalfa can be inoculated after planting. The middle and right plots were inoculated several months after planting, showing that perennial legumes can be rescued from nitrogen starvation. Annual legumes have too short of a growing season for this to work with them.

20. M4/9 Intercropping. The legume *Dolichos lablab* is used as an intercrop in this banana orchard in Honduras. It contributes nitrogen to the soil upon later incorporation and provides erosion control.

21. M5/1 The Slurry Inoculation Method. The following slides give an example of seed coating by the slurry method. First, measure corn syrup.

22. M5/2 Add peat based inoculant.

23. M5/3 Stir the mixture until a uniform slurry results.

24. M5/4 Measure seed into a roomy bucket.

25. M5/5 The slurry is added to the seeds which are stirred until seeds are well coated.

26. M5/6 Optionally, lime may be used for a protective coating after seed inoculation.

27. M5/7 A measured amount of lime is added to the seeds until they are uniformly coated.

28. M5/8 Seed Coating By the 2-Step Method. First, a measured amount of sticker material is added to seeds contained in a plastic bag. Then, the plastic bag is closed in such a way that as much air as possible is trapped in the bag. Vigorously shake the bag for one minute to uniformly wet the seeds with sticker. Next, the peat inoculant is added to the sticky seeds. Again close the bag and shake gently for another minute to coat the seeds with inoculant. Finally, the coated seeds are poured onto a clean surface, spread out and allowed to dry.

29. M6/7 Comparison Table. Soybean cultivation affects the number of soybean rhizobia in soil.

30. M6/7-2 Graphic: A Response Model. Factors controlling the response farmers can obtain by inoculating their legumes.

31. M6/7-3 Comparison of Nodule Amounts From Inoculated and Uninoculated Plants. Inoculation can increase the number of nodules on legumes. On the right, nodules from a system having poor nodulation on uninoculated legumes. Note the few, but relatively large nodules.

32. M6/7-2 Field Study View. Response to inoculation is evident by the size and color of plants.

33. M6/7-5 Starter N Benefit Chart. The benefits to starter nitrogen are a function of both the legume and the soil.

34. M8/1 You Are The Key. Introduce the concept of responsibility with this slide pointing out that each participant is responsible for their role in the BNF technology transfer process.

A series of self-explanatory text slides follows:

Communication & Teaching Skills:

35. M8/2 What Motivates People to Learn?

36. M8/3 What Adult Learners Expect

37. M8/4 How People Learn

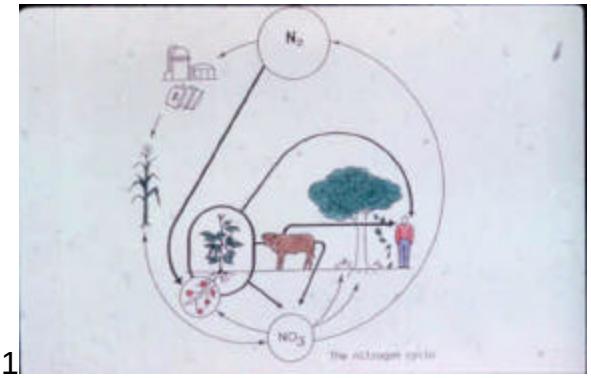
38. M8/5 People Remember

39. M8/6 Teaching Method

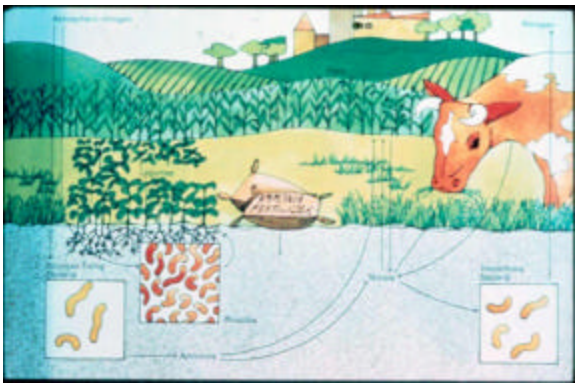
Planning Technology Transfer:

40. M817 When Planning, Systematically consider

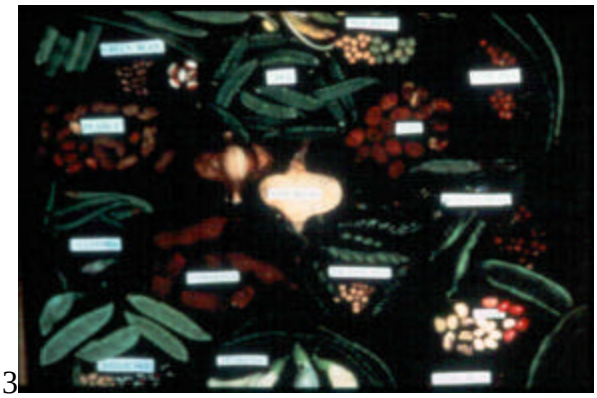
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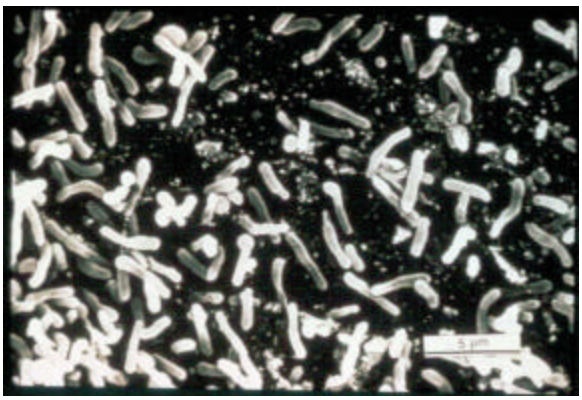
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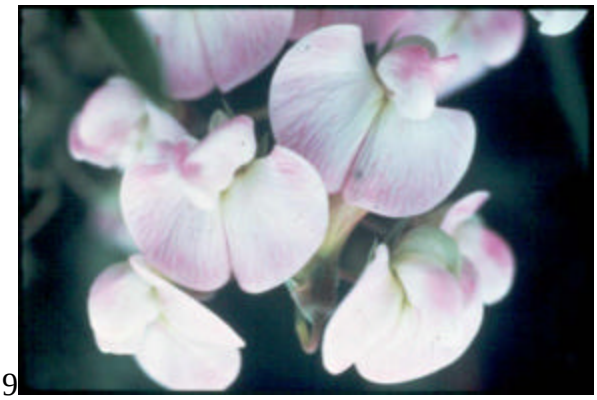


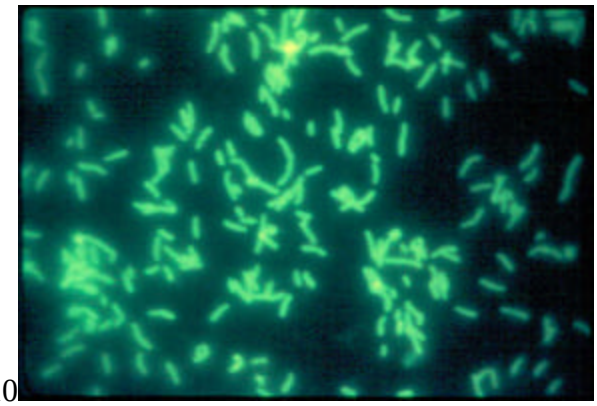
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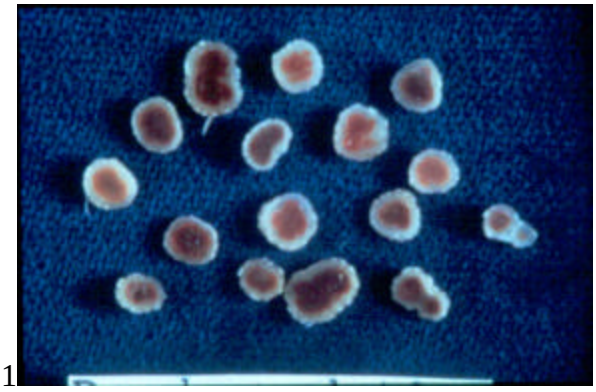


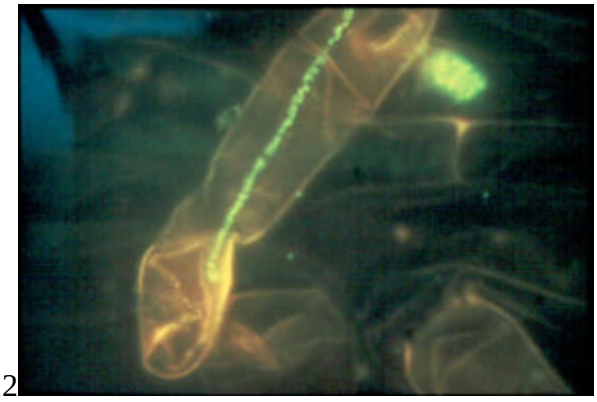
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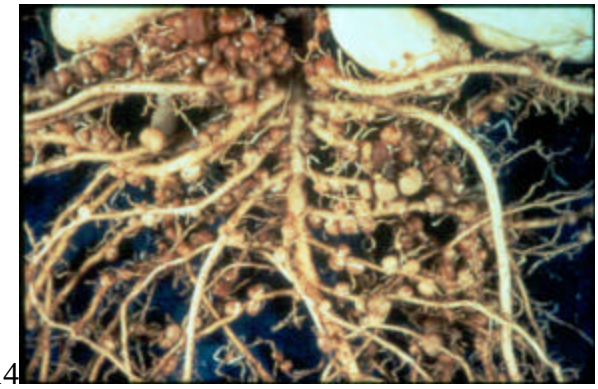
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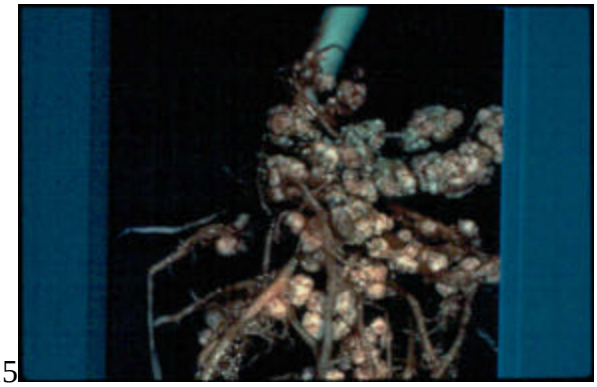
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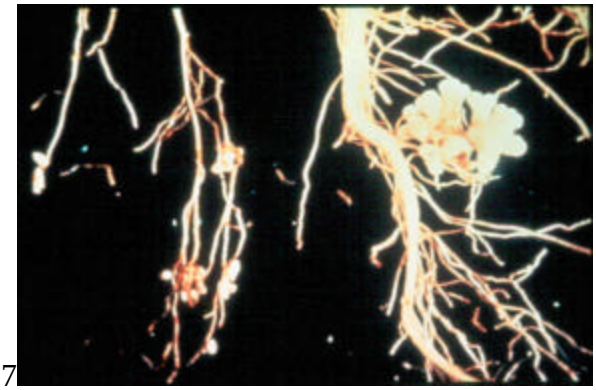
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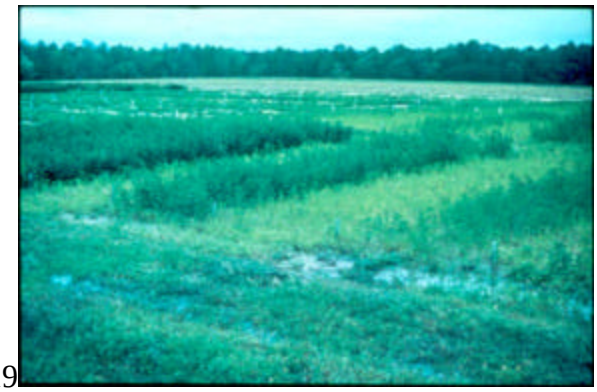
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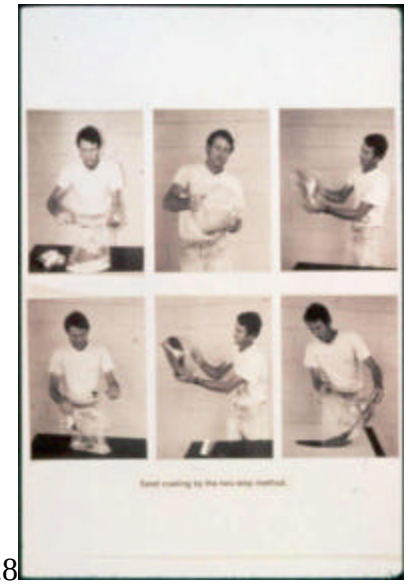
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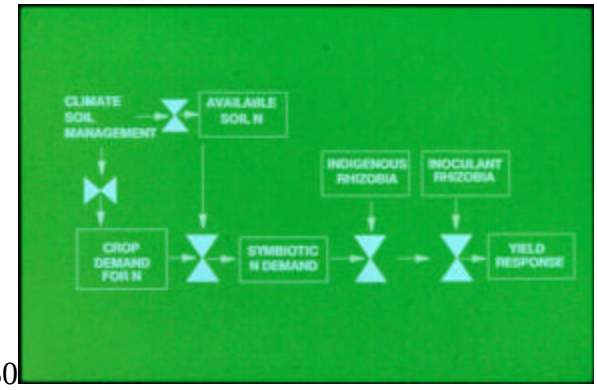
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Frequency Distributions of *Bradyrhizobium japonicum* populations in Sumatra in Relation to the Number of Years of Soybean Cultivation.

Number	Years 0	1	2-5	>5
g^{-1} soil	relatively frequency (%)			
0-10	61	60	0	0
10-100	21	40	33	27
100-1000	7	0	33	27
>1000	11	0	33	45

28, 5, 6, 11 observations for 0, 1, 2-5, >5 years.

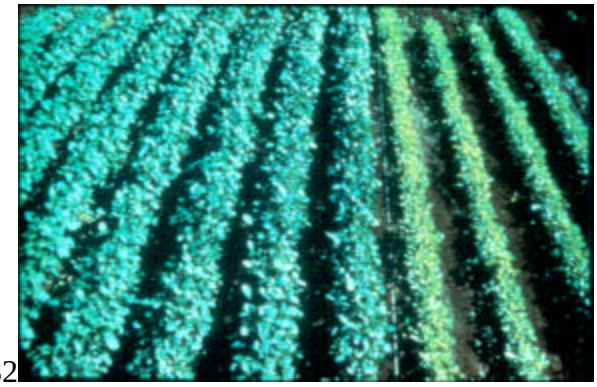
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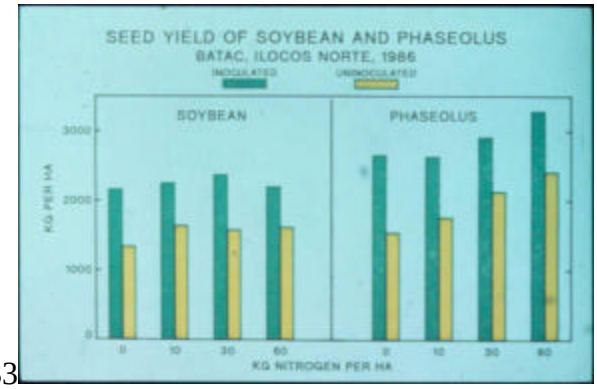
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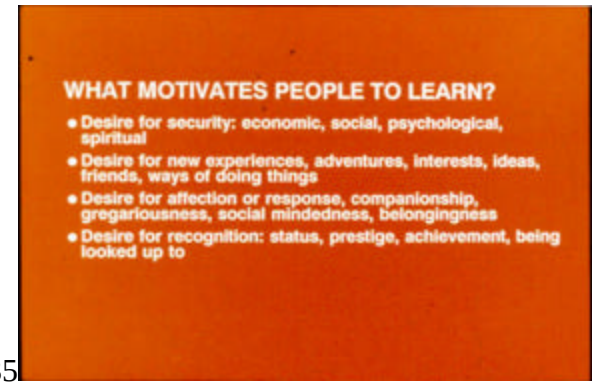
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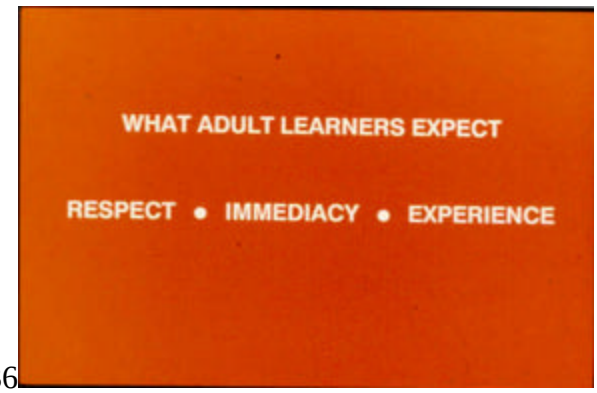
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HOW PEOPLE LEARN

4 Important Requirements To Help Adults Learn Best:

- When they have a strong desire to learn
- When they put into practice what they have learned
- When they have clear objectives or goals
- When they experience satisfaction from what they have learned

38

PEOPLE REMEMBER

- 20% OF WHAT THEY HEAR
- 40% OF WHAT THEY HEAR AND SEE
- 80% OF WHAT THEY DO

39

TEACHING METHOD:

SHOW IT



TELL IT



DO IT



40

WHEN PLANNING,
SYSTEMATICALLY CONSIDER:

WHO
WHY
WHEN
WHAT FOR
WHERE
WHAT
HOW