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.

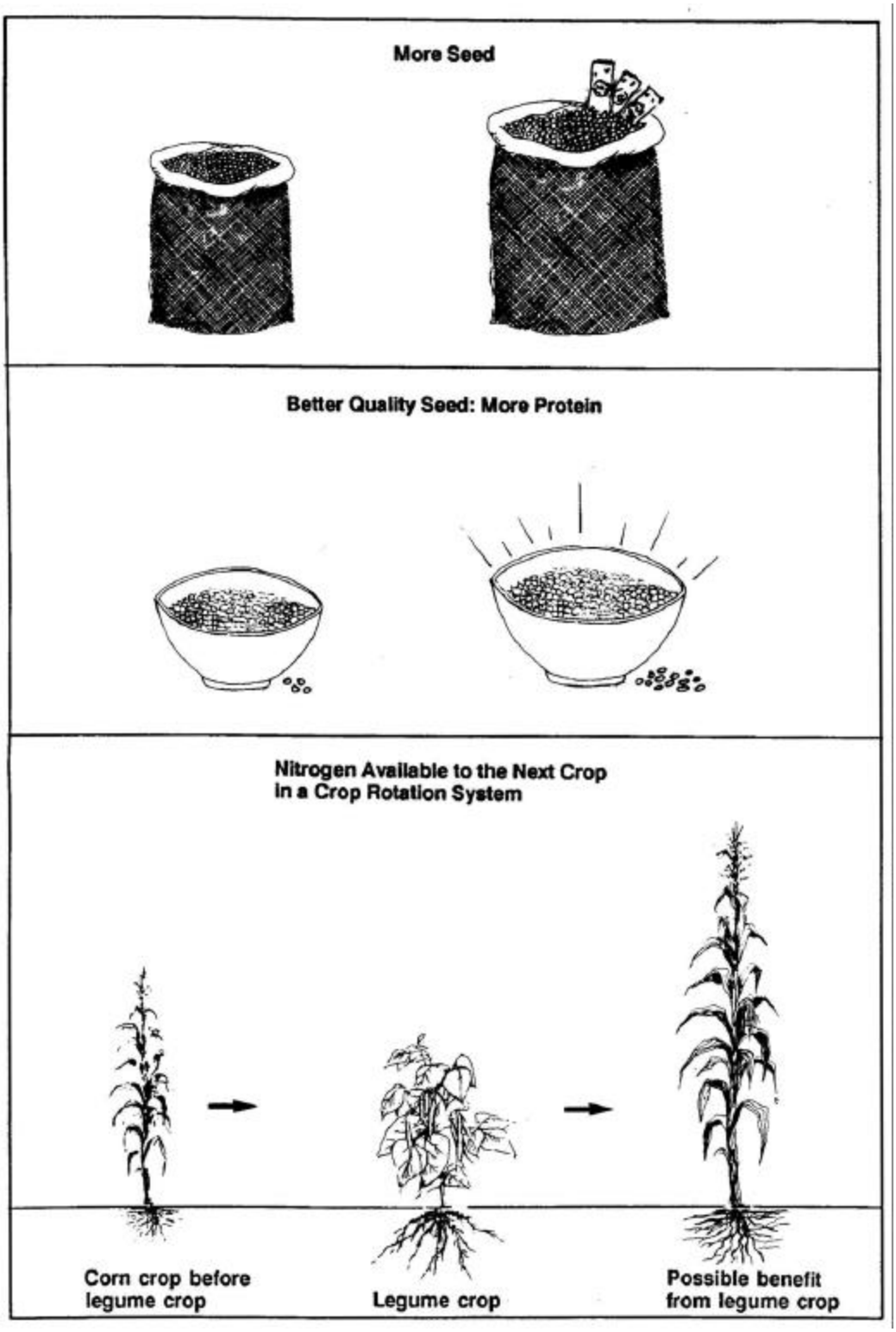
More Soil Nitrogen Available for Other Crops

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.

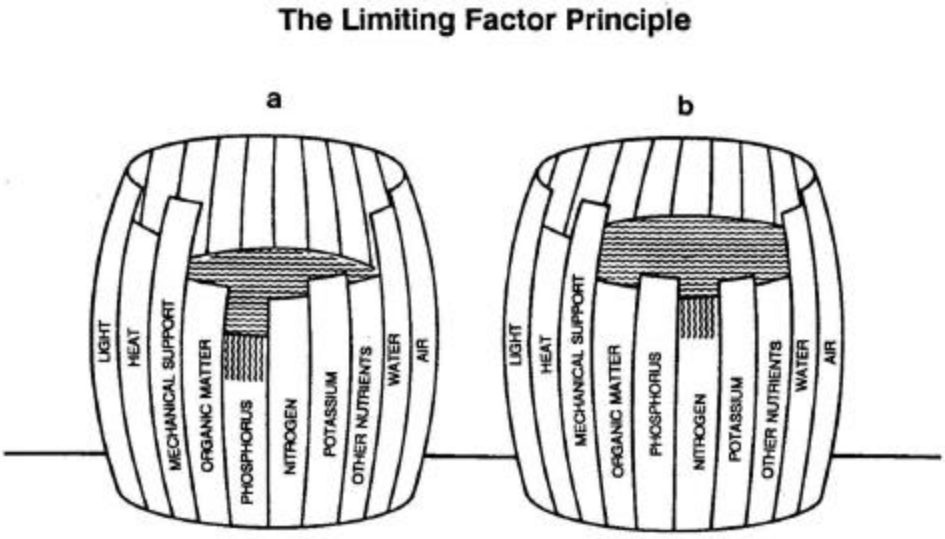


Figure 6-2. Nutrient limitations are important considerations in The Law of the Minimum.

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.

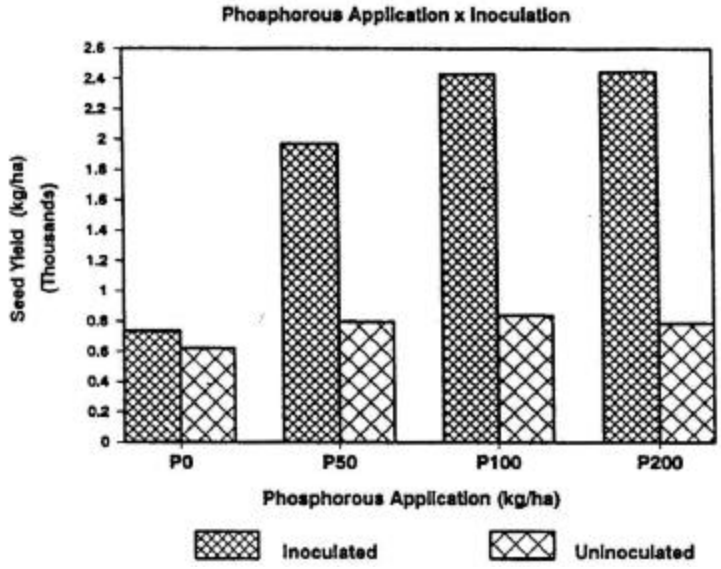


Figure 6-3. Phosphorus deficient soil limits response to inoculation.

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.

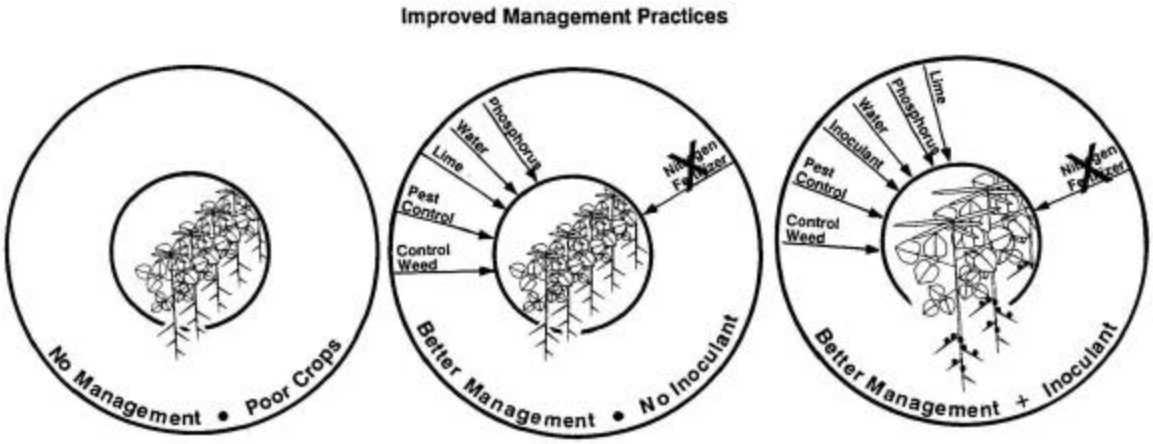


Figure 6-4. Good management practices ensure good crops and benefits from legume inoculation.

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.

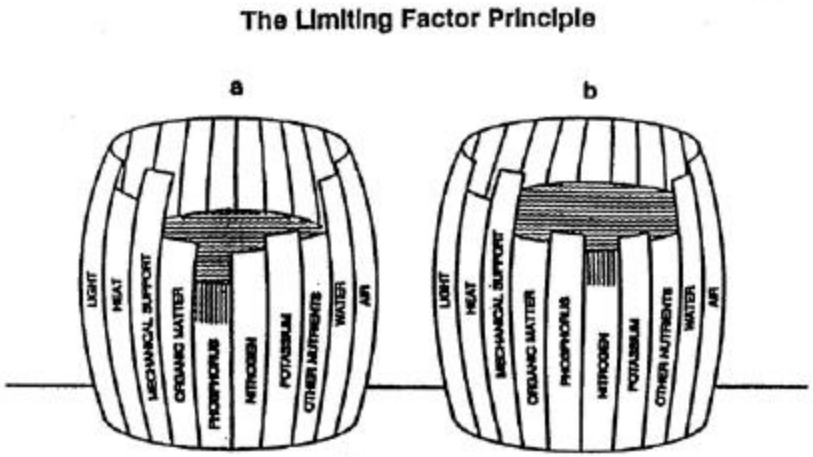
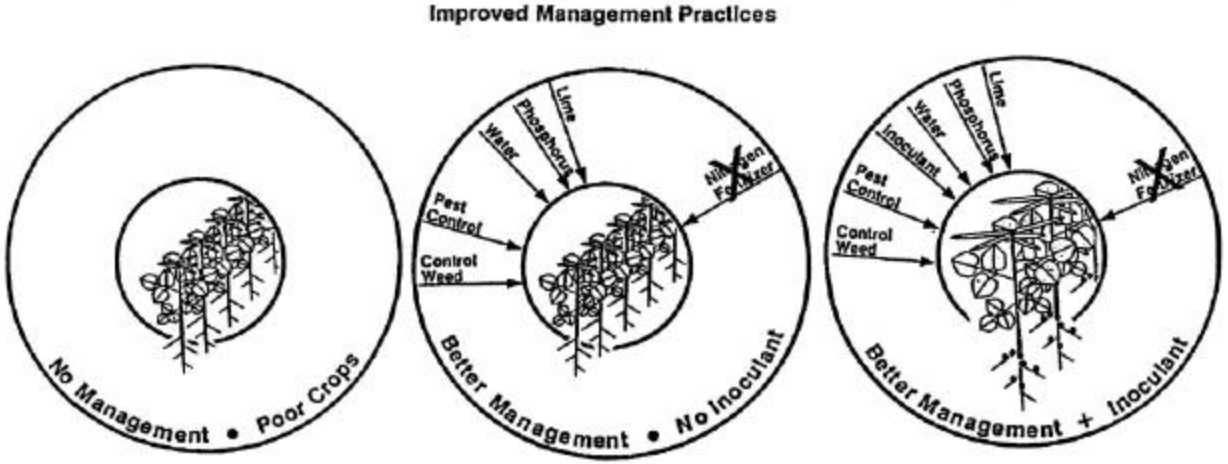
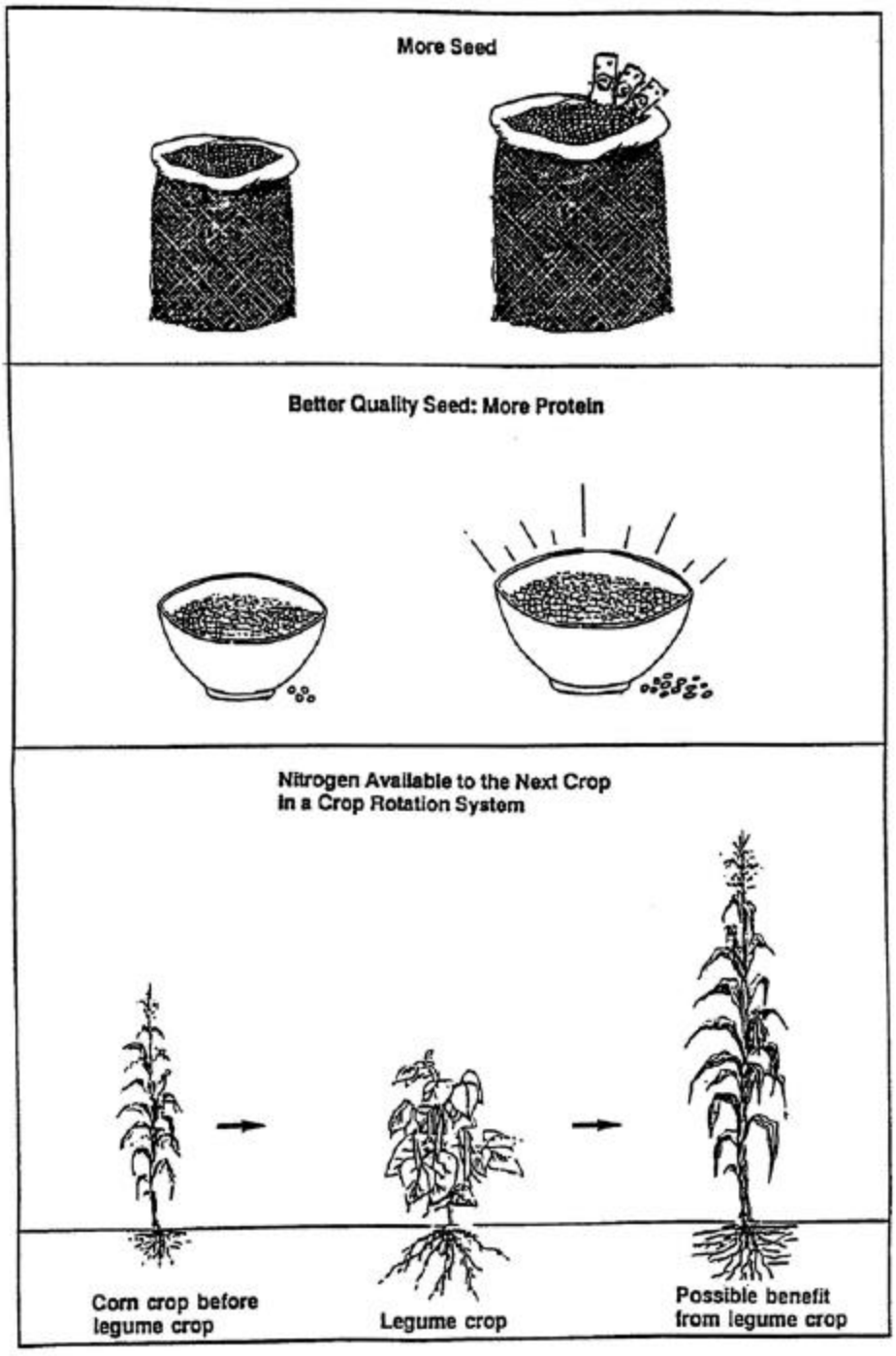


Figure 6-2. Environmental factors and nutrient limitations are important considerations in the Law of the Minimum.




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