

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 μm wide and 1.2 to 3.0 μm long. Like most living things, they need a supply of air (oxygen) to live. They can move using special thread-like structures called flagella.

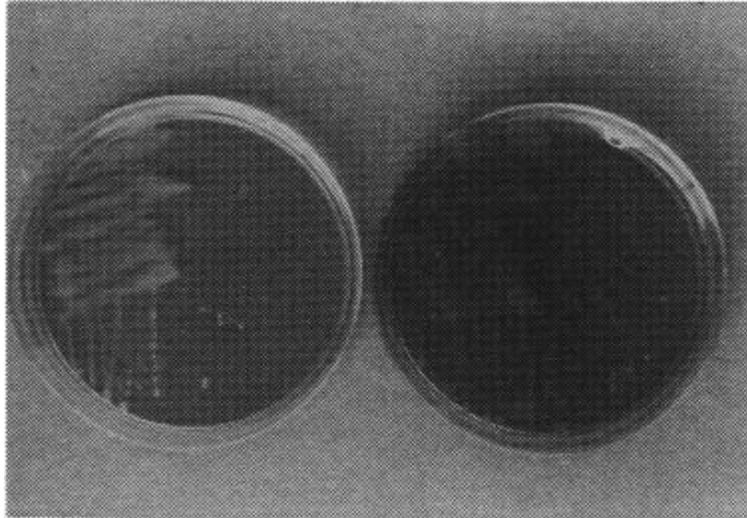


Figure 3-1. Rhizobia grown on YMA media have a gummy or slimy appearance.

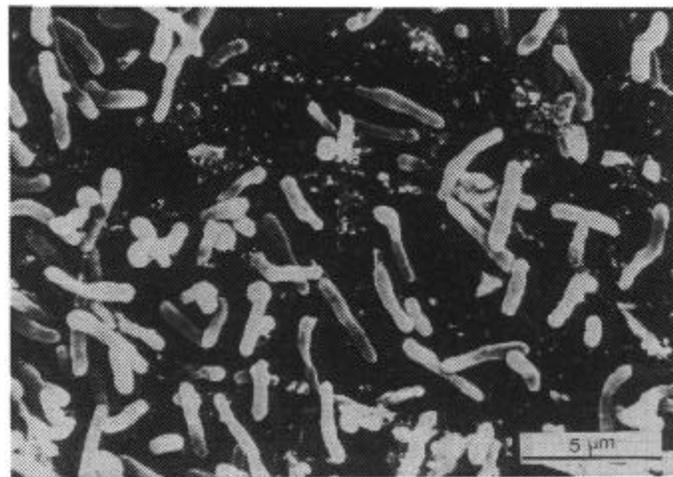


Figure 3-2. Rod shaped rhizobia in the nodule of cowpea (*Vigna unguiculata*).

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**.

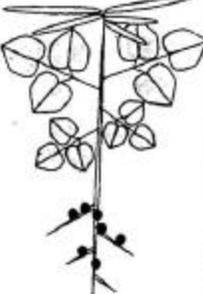
Table 3-1 shows that we should not inoculate soybean with pea rhizobia, for instance, or leucaena with soybean rhizobia. What happens if we do? In some cases, no nodules will form. This means that there will be no nitrogen fixation to benefit the legume. In other cases, nodules may form, but they will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

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

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.

Name of Rhizobia	Legume Crop			
	Soybean (<i>Glycine max</i>)	Peanut (<i>Arachis hypogaea</i>)	Mungbean (<i>Vigna spp.</i>)	Leucaena (<i>Leucaena leucocephala</i>)
Soybean (<i>Bradyrhizobium japonicum</i>)				
Cowpea (<i>Bradyrhizobium spp.</i>)				
Leucaena (<i>Rhizobium sp.</i>)				

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 production. 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, than 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).

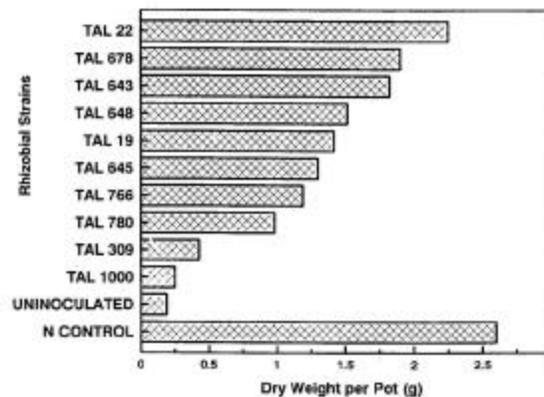


Figure 3-4. Effect of inoculation with strains of rhizobia on shoot dry weights of rice bean (*Vigna umbellata*) grown in the greenhouse at NifTAL, Maui, Hawaii.

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
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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
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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).

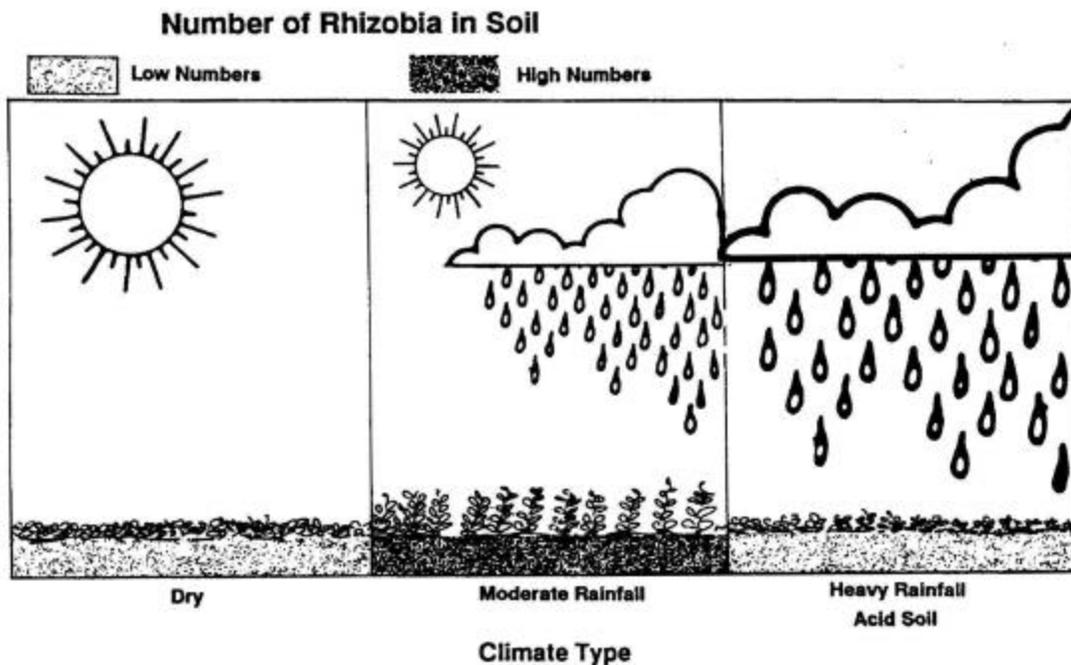


Figure 3-5. The effect of climate on rhizobial populations.

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.

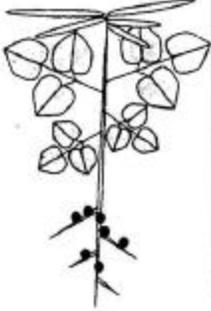
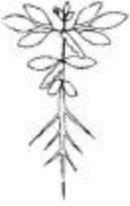
Rhizobial inoculants must be properly matched with the legume.

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

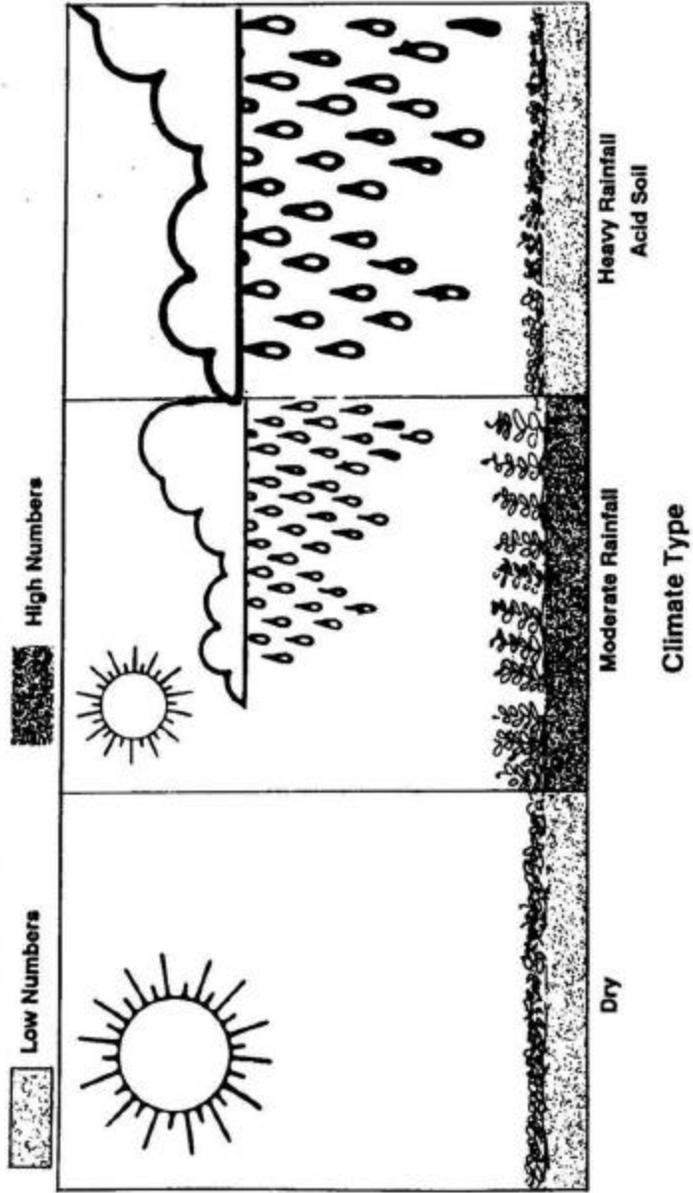
The soil and environment affecting native and introduced rhizobia in the soil.

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.

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Cowpea (<i>Bradyrhizobium spp.</i>)				
Leucaena (<i>Rhizobium sp.</i>)				

Number of Rhizobia in Soil



MODULE 3

