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.

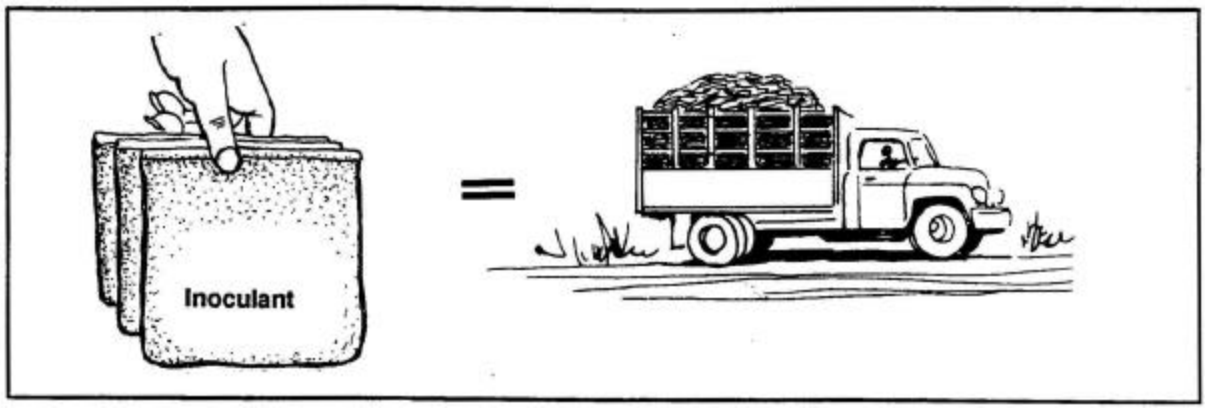


Figure 5-1. The truck contains 4 tons of soil from a field which had produced well nodulated legumes. the hand holds three 100 gram bags of rhizobia inoculant. There are the same number of living rhizobia in the three small bags as in the truck filled with soil.

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.

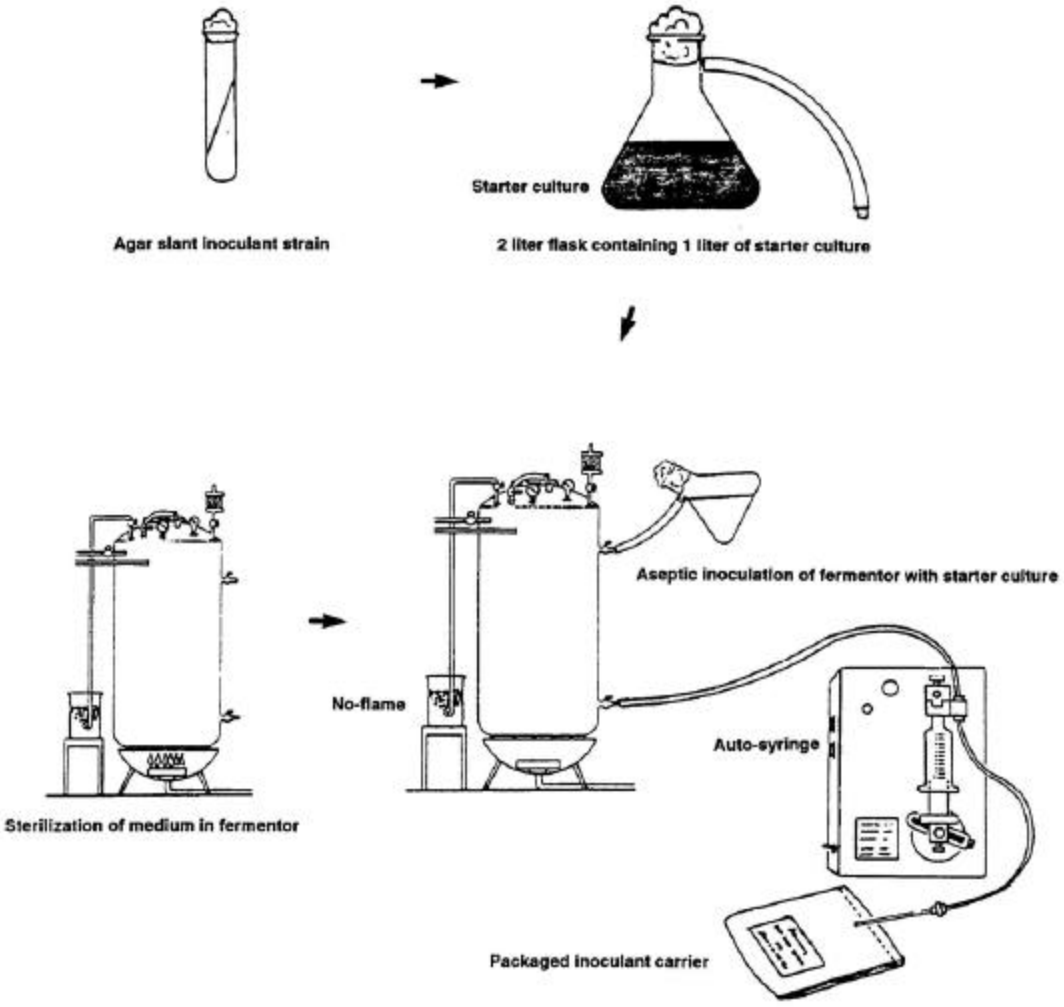
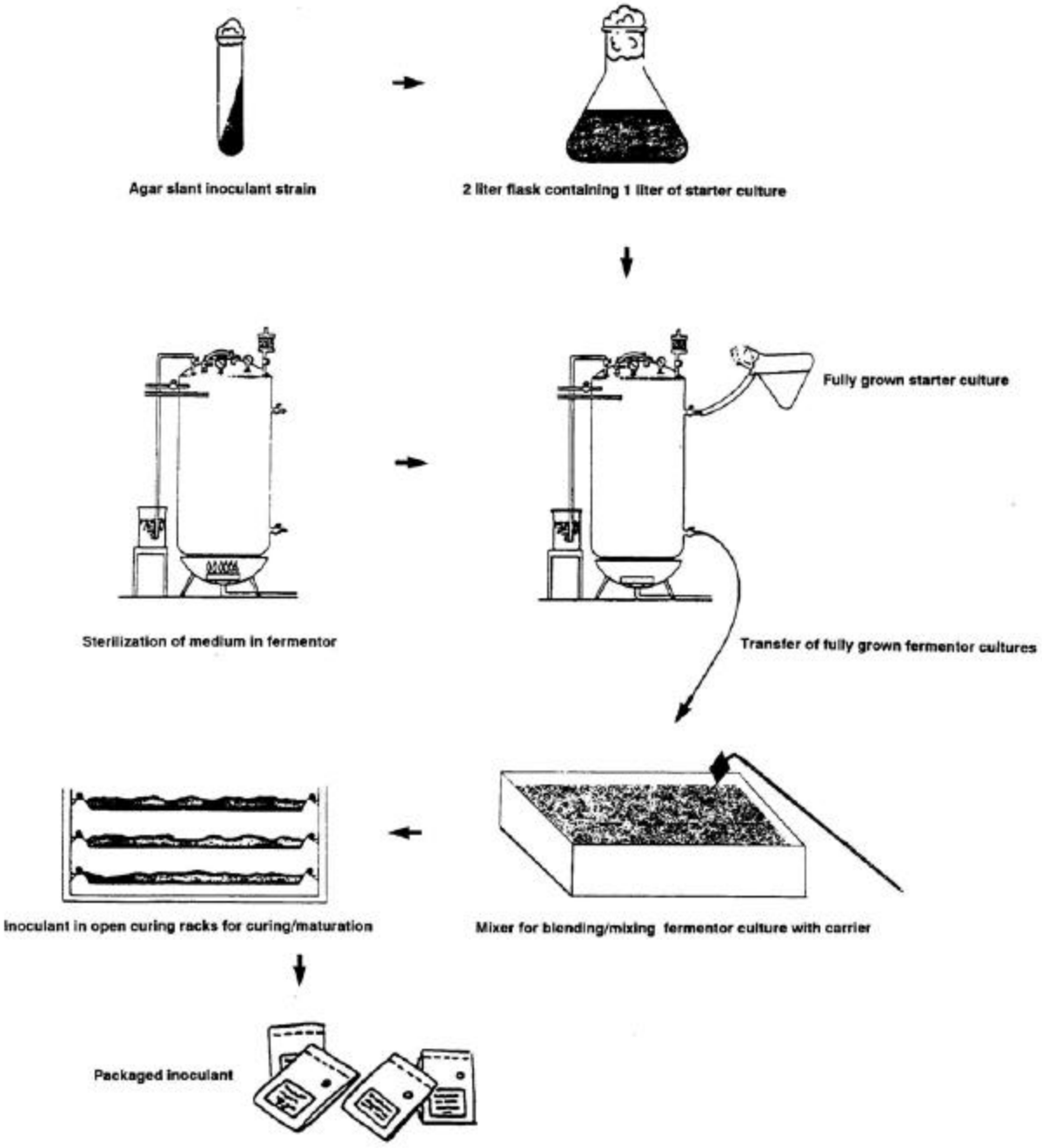


Figure 5-3. Inoculant production using non-sterile carrier.



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.

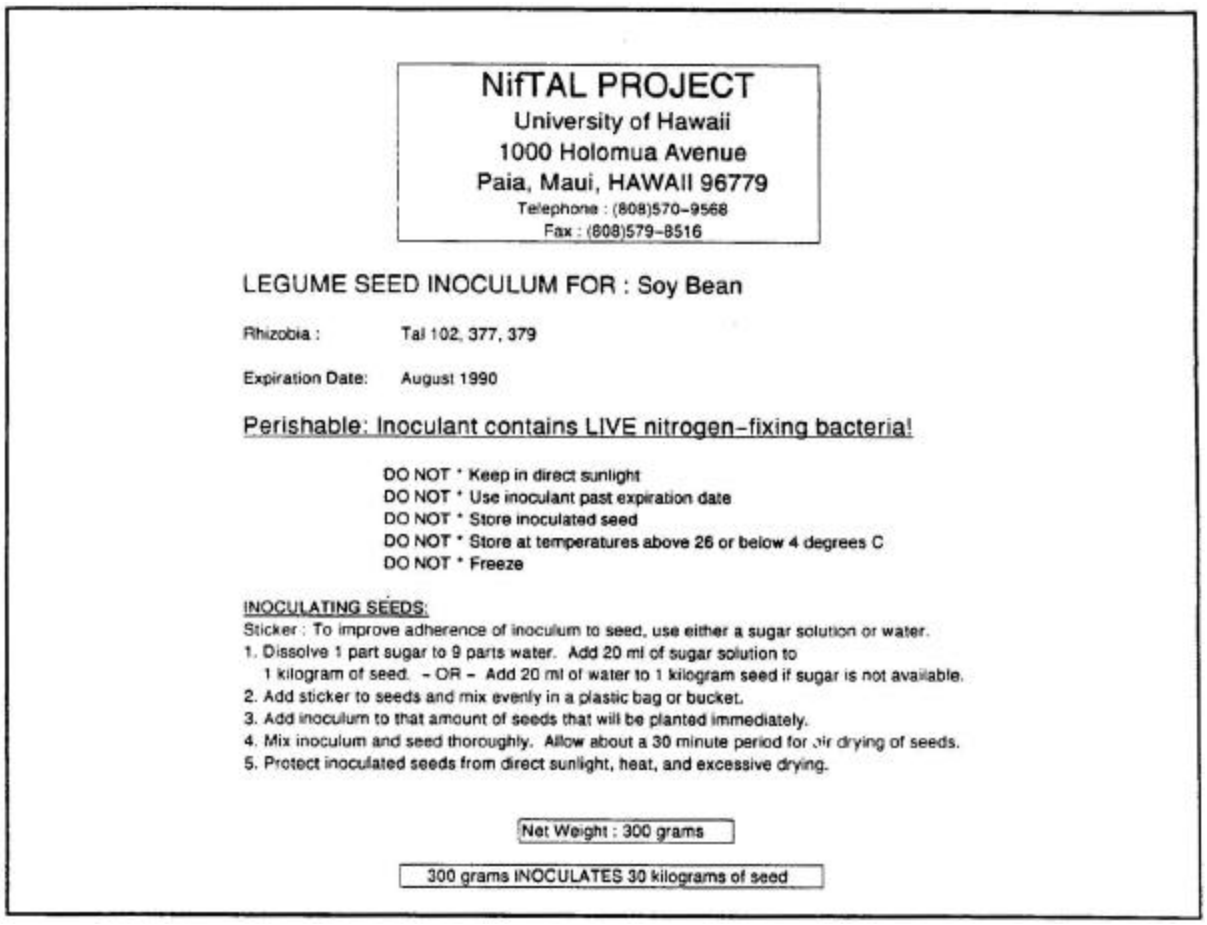
3. **Tolerant of stress**. It must stimulate nodulation and nitrogen fixation over a wide range of soil temperatures, acidity, alkalinity, and salinity conditions.

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.



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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.

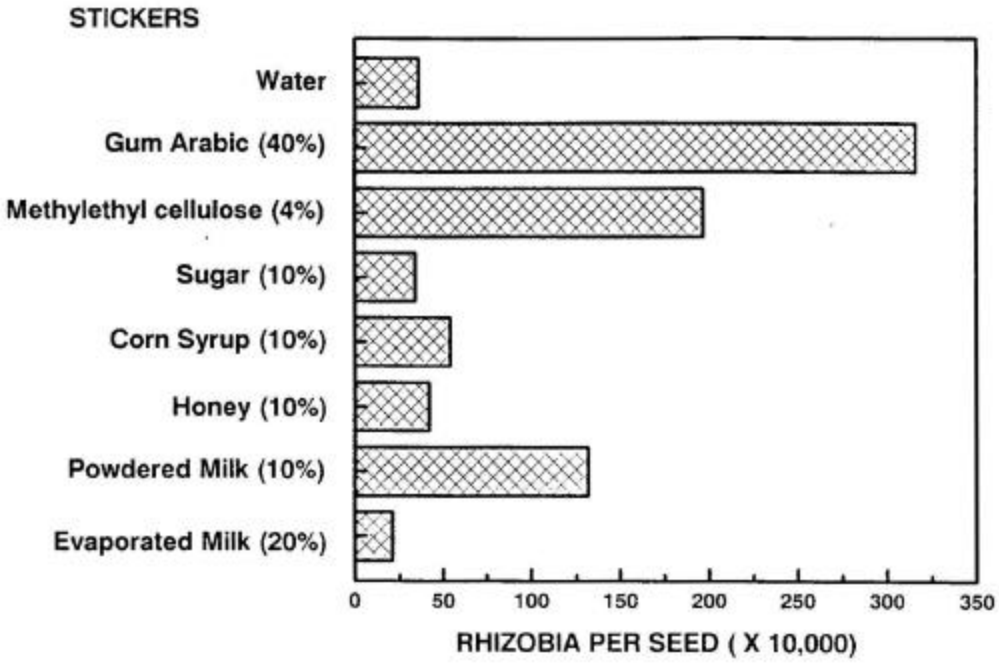


Figure 5-5. Effect of different stickers on the number of viable inoculant rhizobia on soybean seeds at time of planting. Adapted from Elegba and Rennie, Can. J. Soil Sci., 1984.

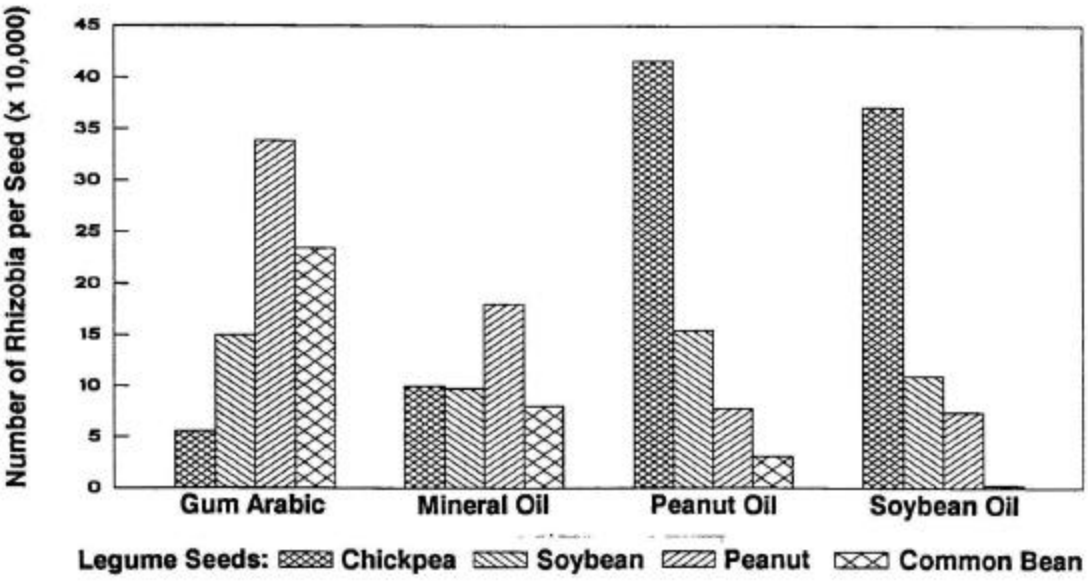


Figure 5-6. Survival of rhizobia on legume seeds after 3 days of storage at 34°C (Hoben, et al, 1991).

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.

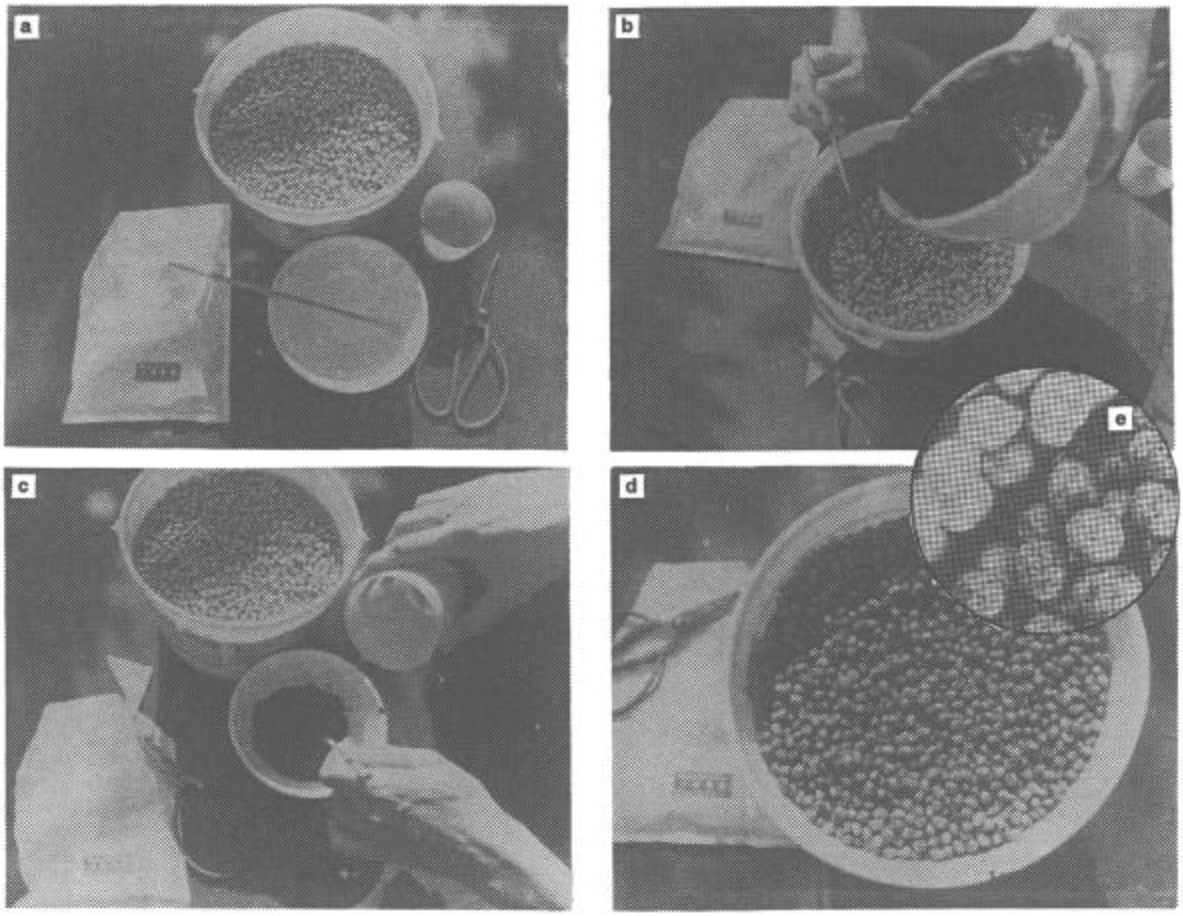


Table 5-3. Amounts of inoculant and sticker solution required for the slurry

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.

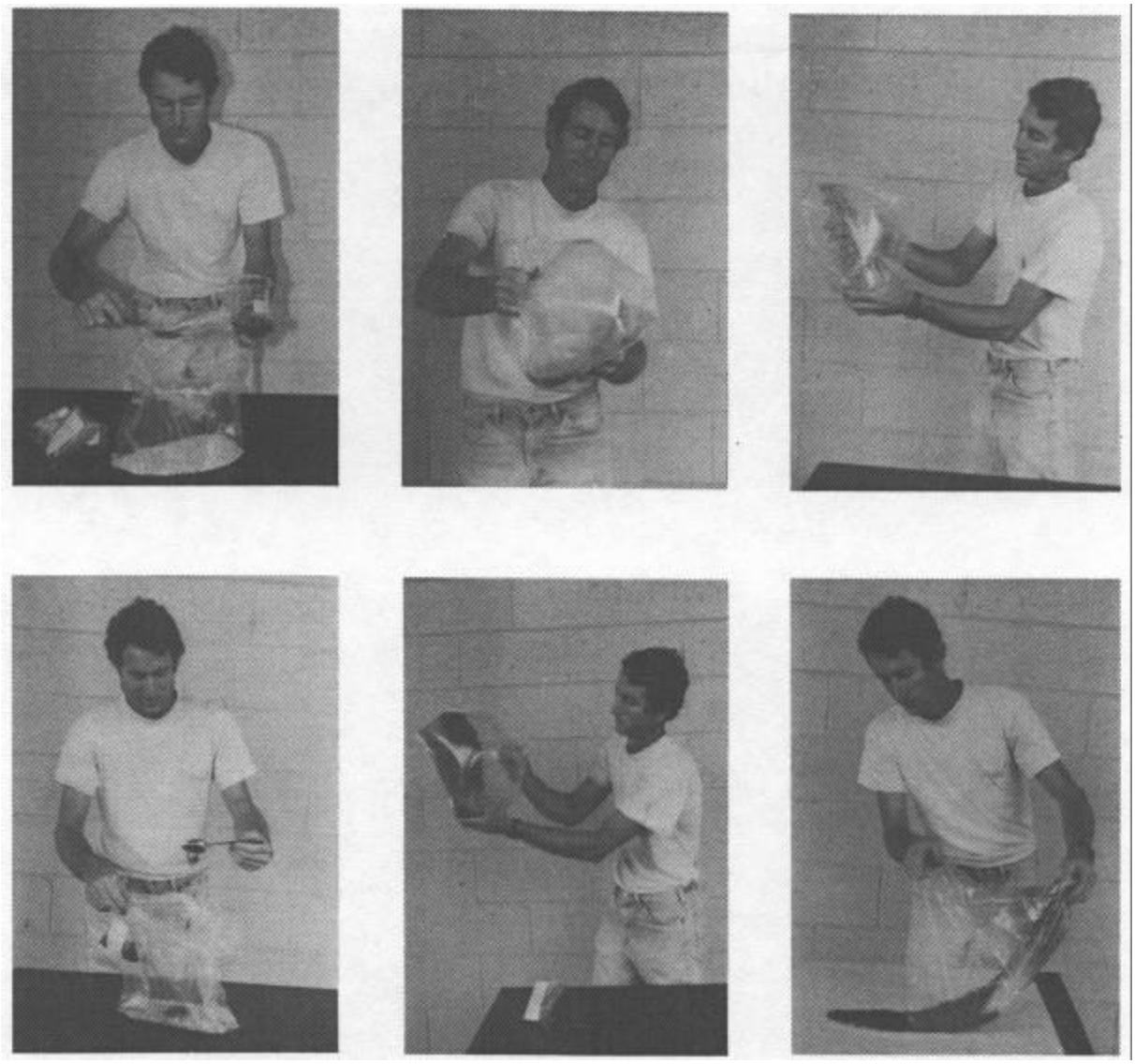


Figure 5-8. Seed coating by the two-step method.

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.

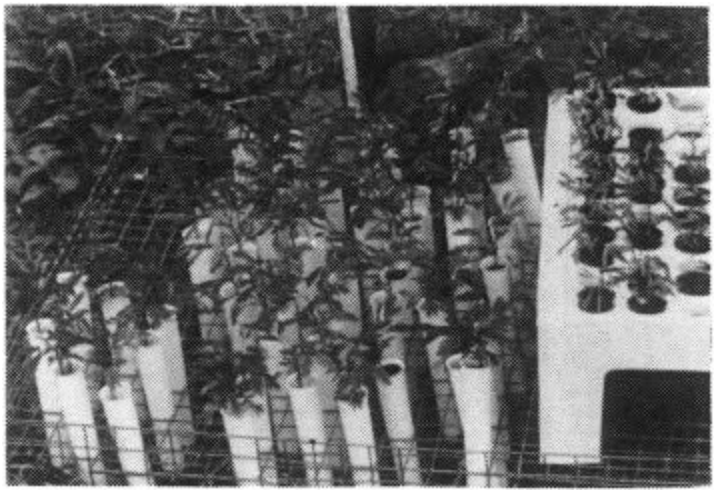


Figure 5-11. These trees were planted in dibble tubes for transplanting.

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.

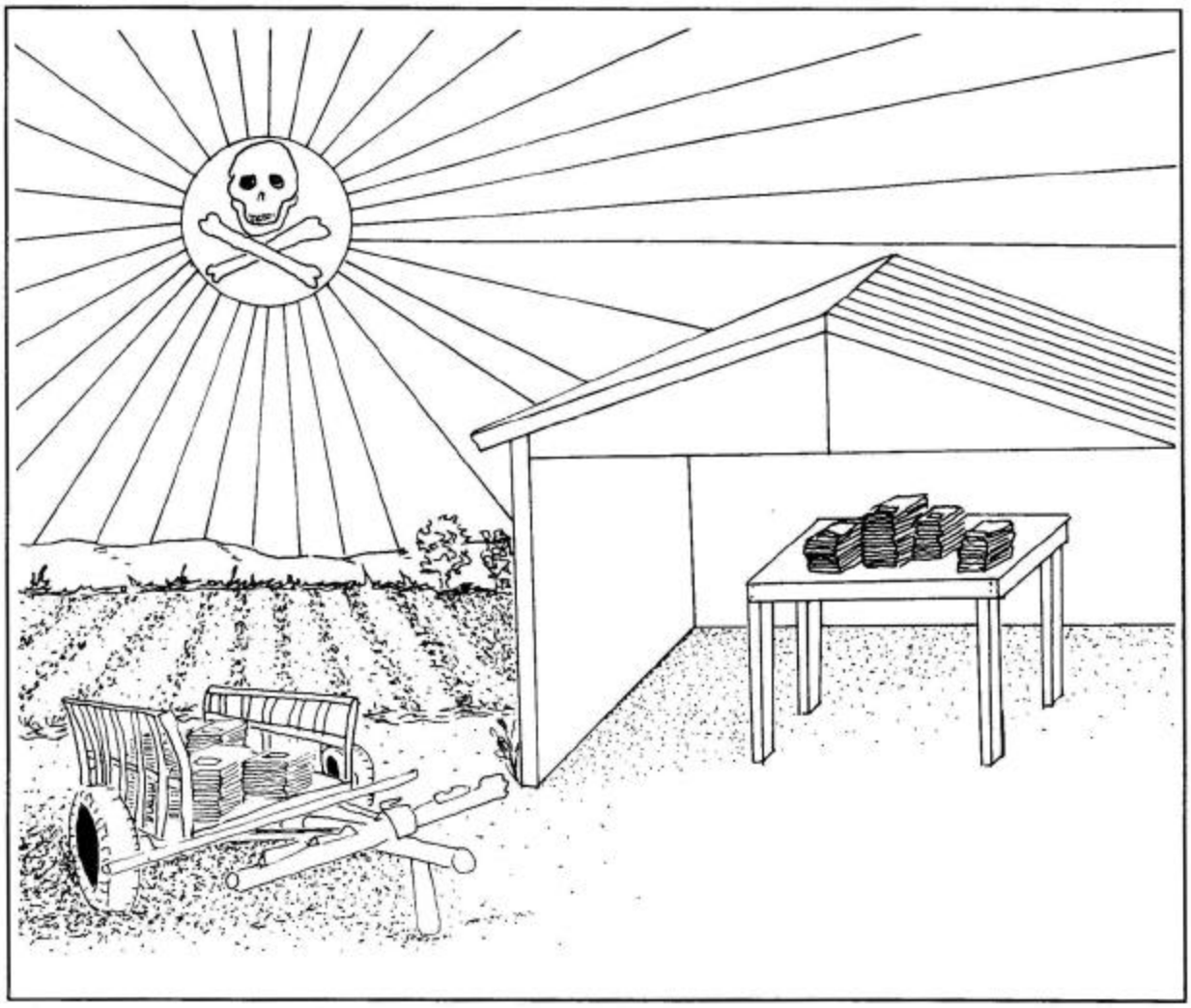


Figure 5-12. It is too hot to store inoculant in direct sunlight or in a metal roofed building.

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.

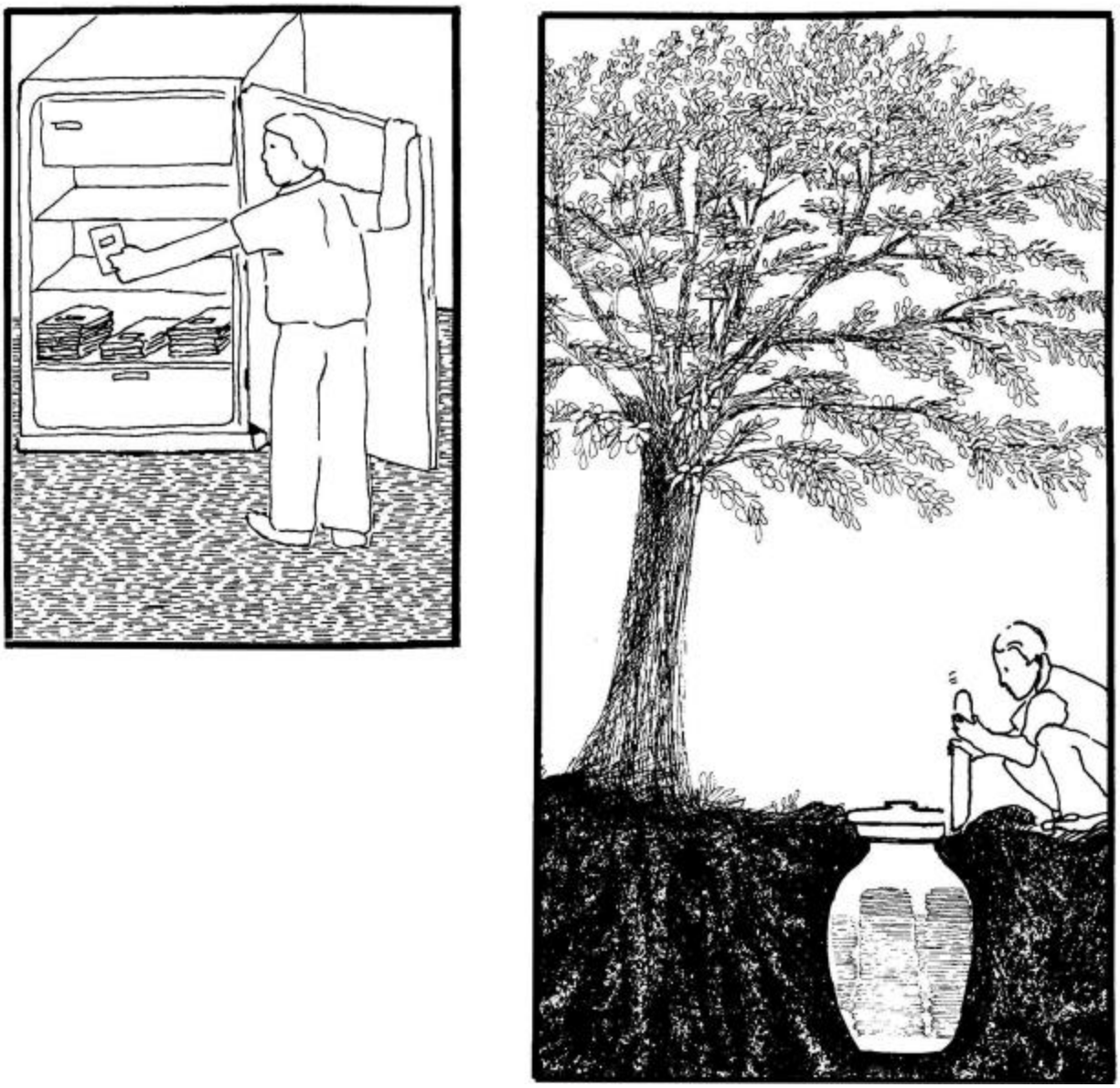


Figure 5-13. It is best to store inoculants in cool places. Refrigeration between 4° and 26° C is ideal. However, using a buried urn in a shady place will allow good storage for at least 6 months.

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.

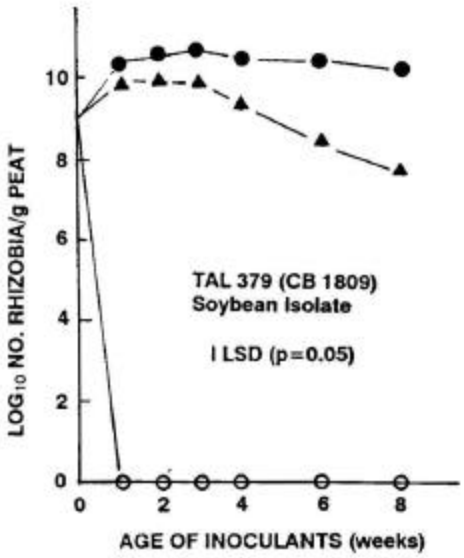


Figure 5-14. Survival of soybean rhizobia at three temperatures.

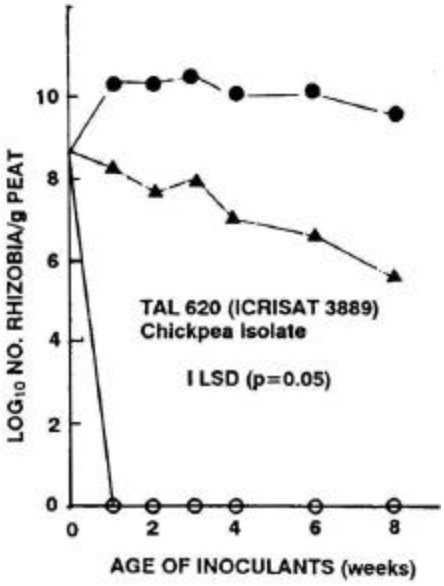


Figure 5-15. Survival of chickpea rhizobia at three temperatures.

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.

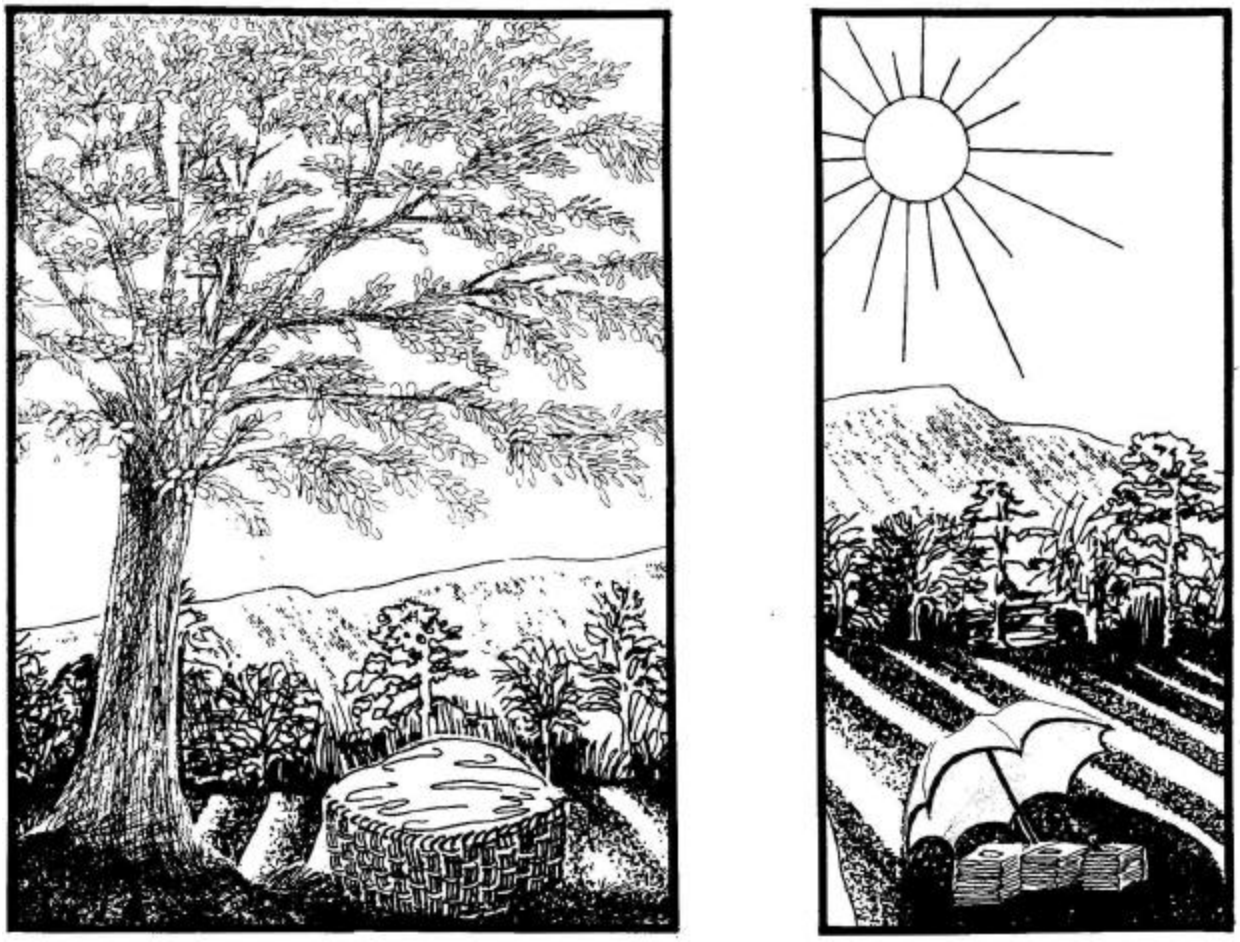


Figure 5-17. This inoculant is protected from the sun by an umbrella. Inoculant may also be placed under a shady tree.

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.

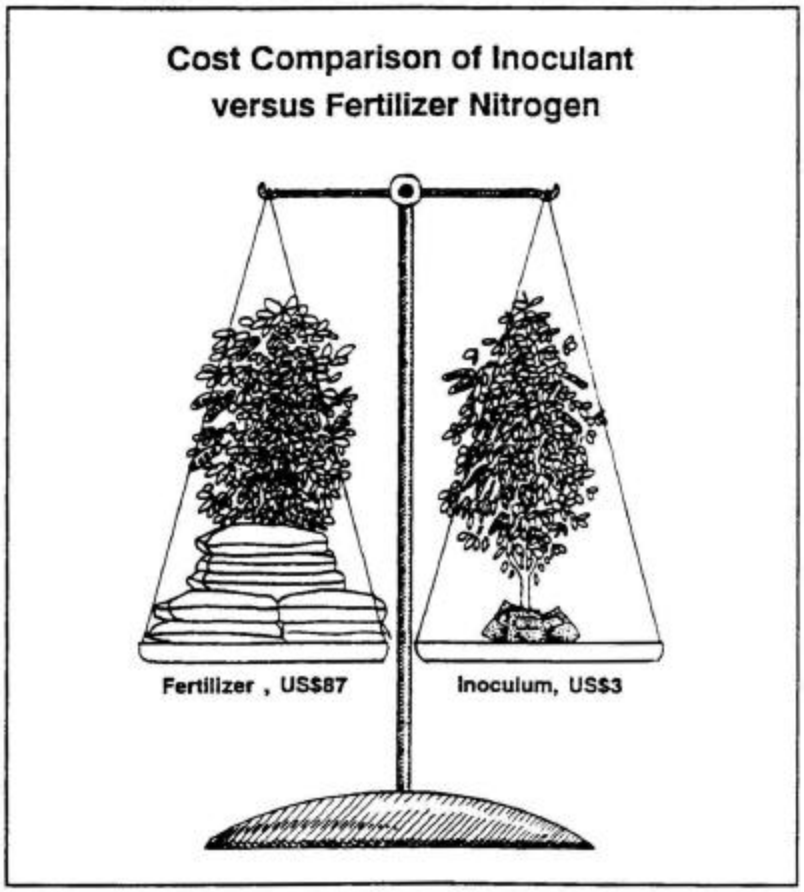


Figure 5-18. This scale shows the dramatic difference between the cost of chemical fertilizers and rhizobial inoculants.

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.



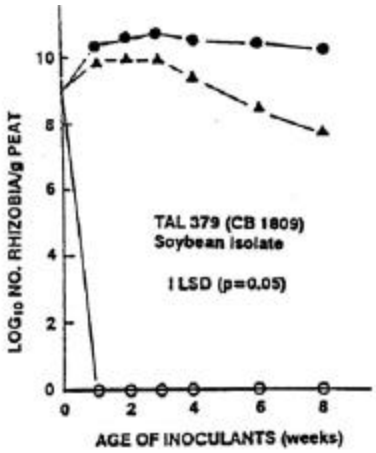


Figure 13. Survival of soybean rhizobia at three temperatures.

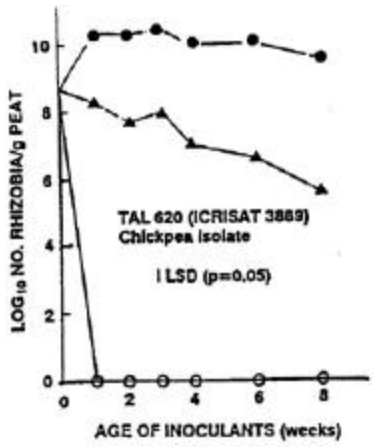


Figure 5-14. Survival of chickpea rhizobia at three temperatures.

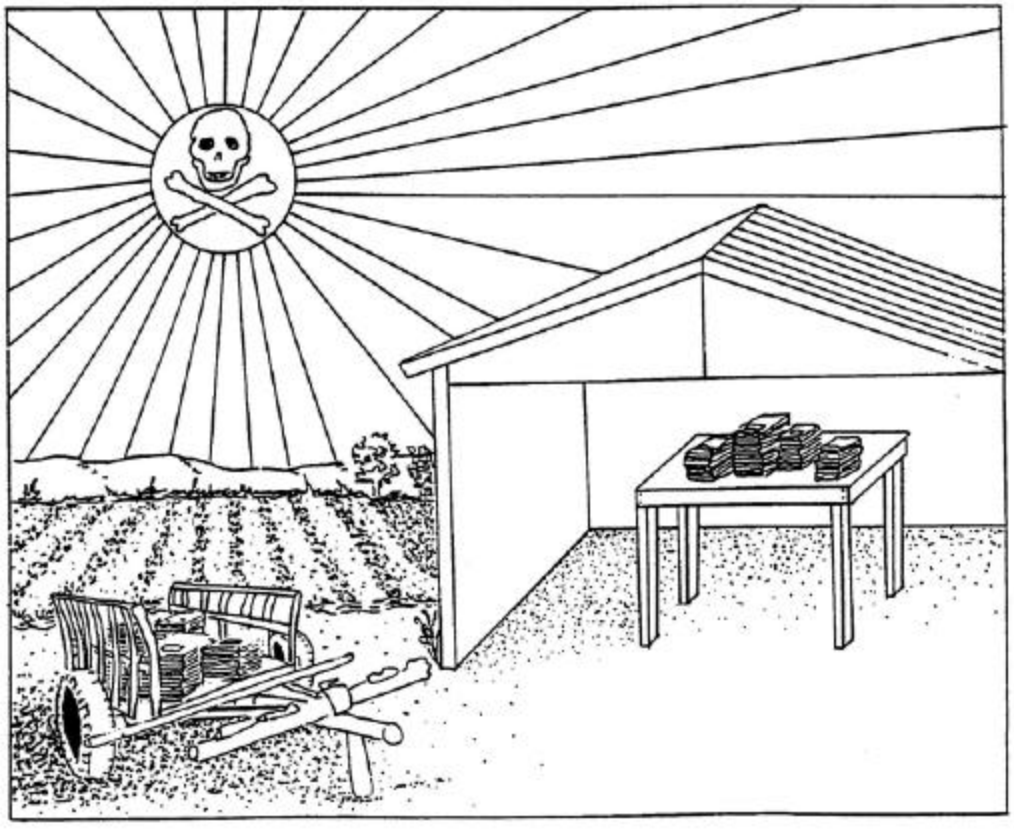


Figure 5-15. Inoculants should be transported to the field in a cool container. These farmers are carrying inoculant in a basket covered with a wet cloth.



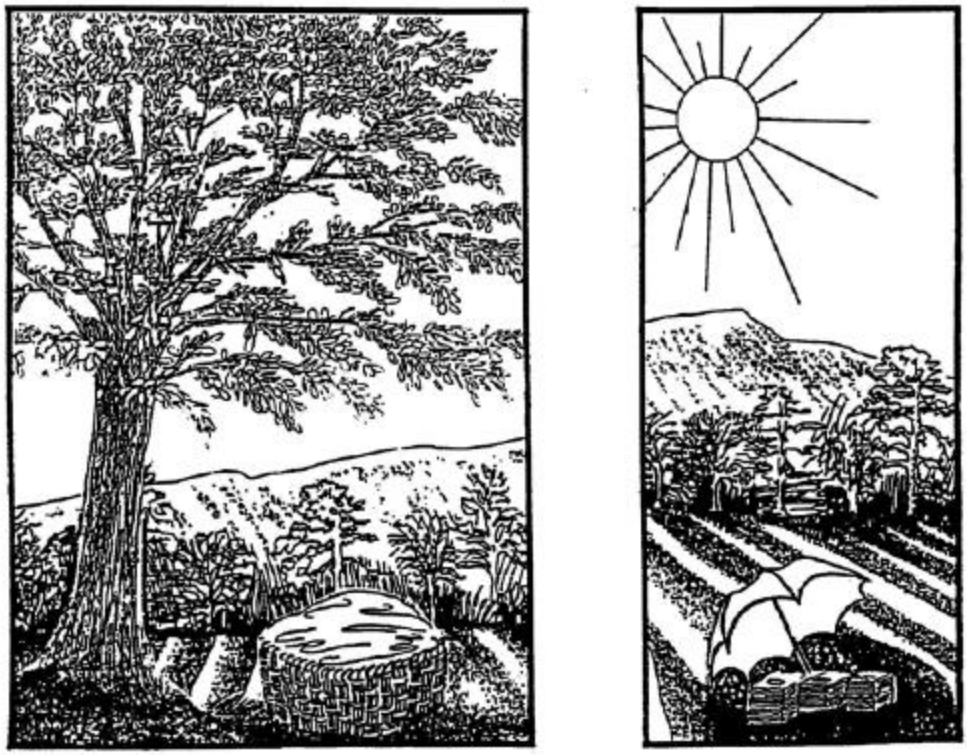


Figure 5-16. This inoculant is protected from the sun by an umbrella. Inoculant may also be placed under a shady tree.

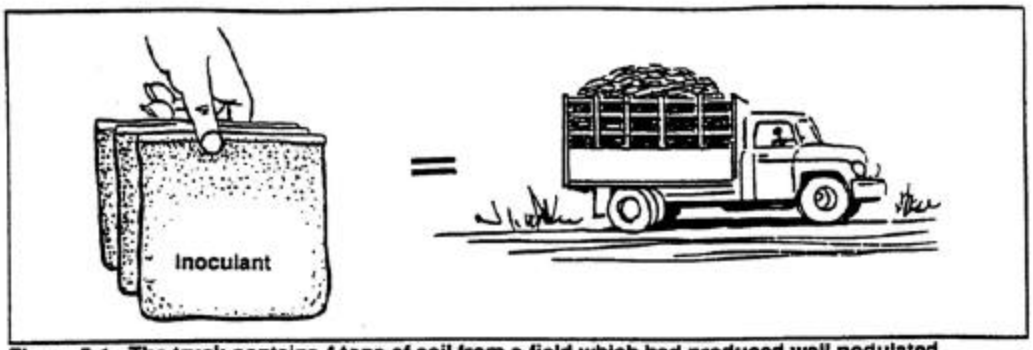


Figure 5-1. The truck contains 4 tons of soil from a field which had produced well nodulated legumes. The hand holds three 100 gram bags of rhizobial inoculant. There are the same number of living rhizobia in the three small bags as in the truck filled with soil.

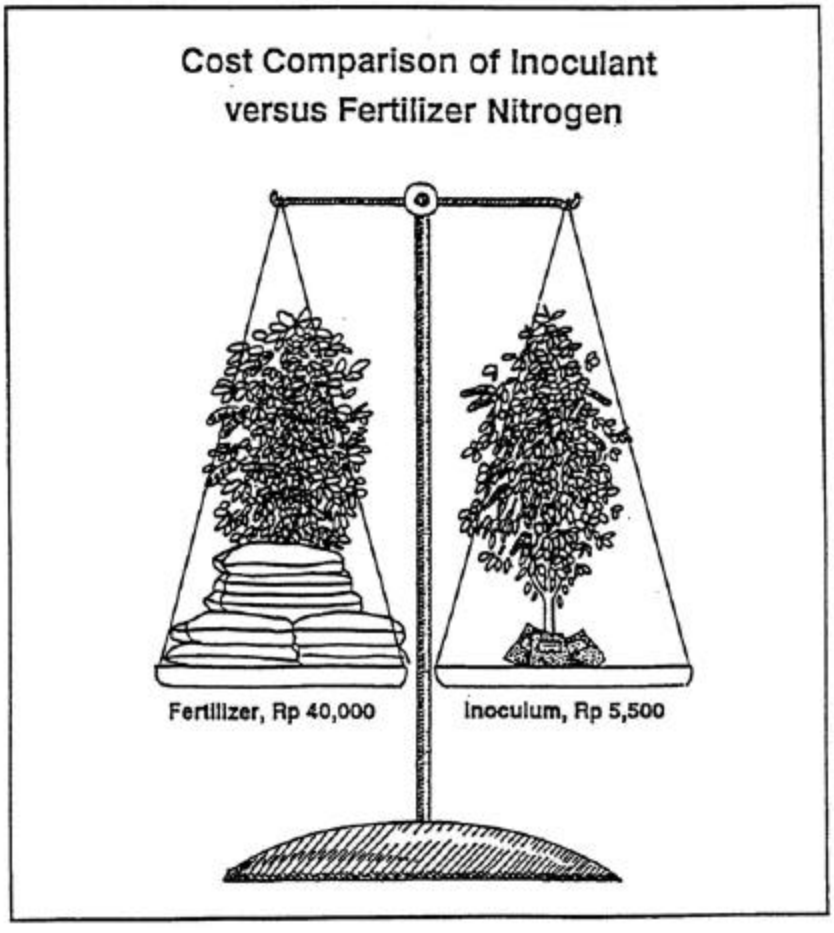


Fig Effect of different stickers on the number of viable inoculant rhizobia on soybean seeds at time of planting.

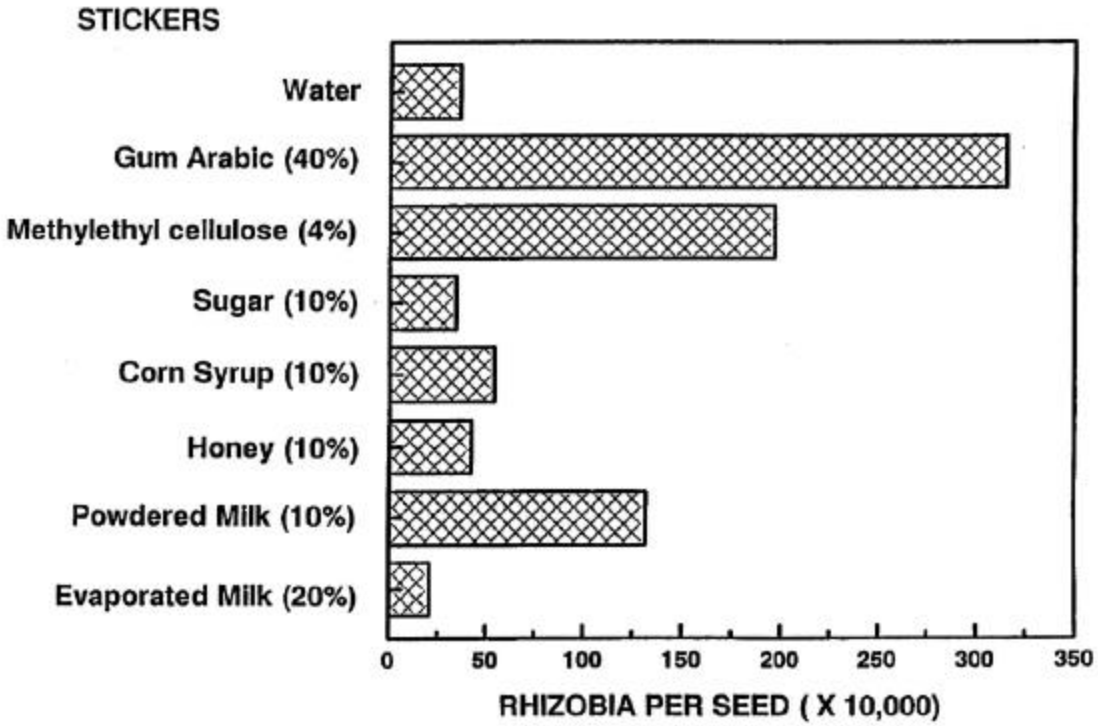
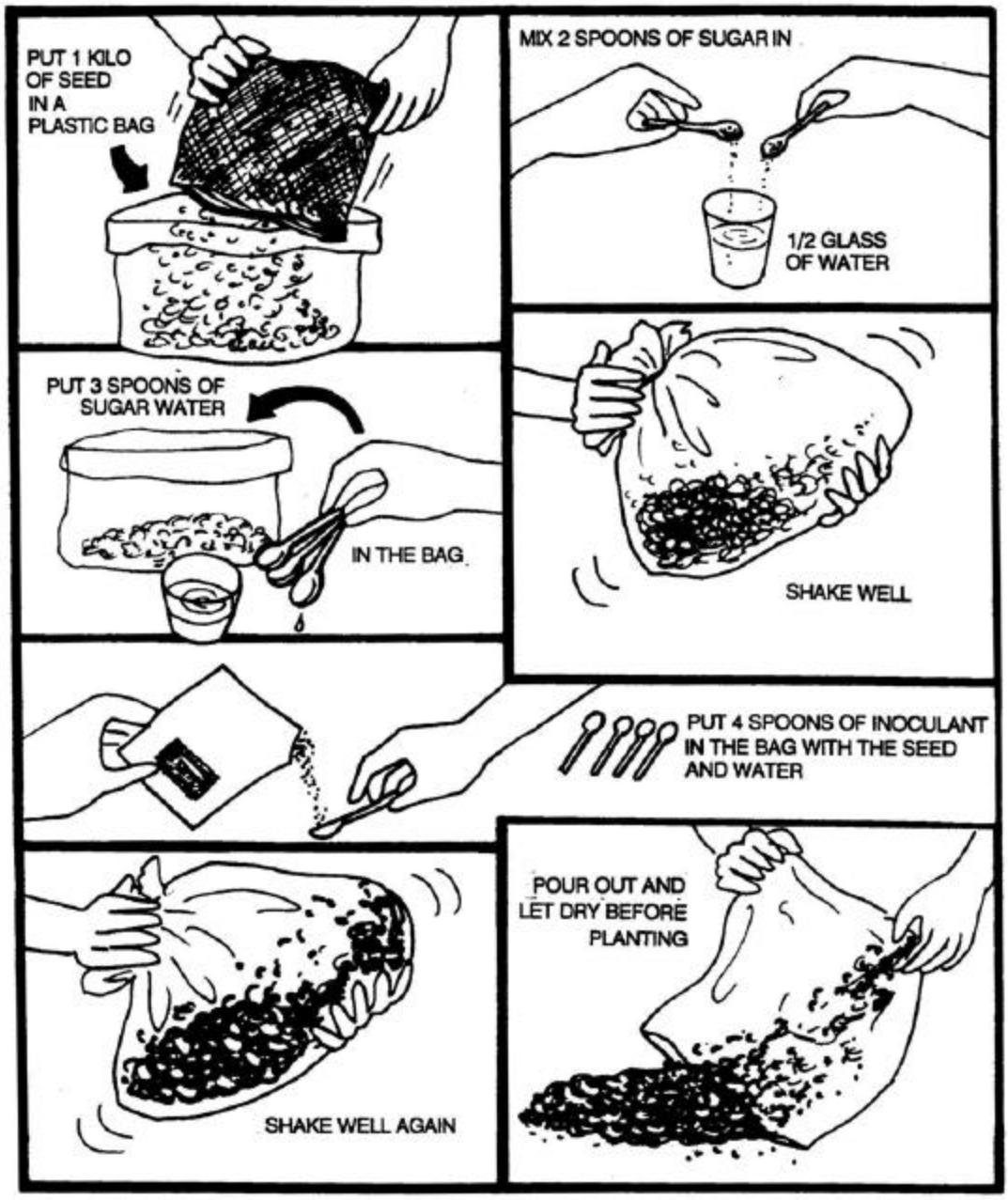






Figure 5-12. It is best to store inoculants in cool places. Refrigeration between 4 and 26 C is ideal. However, using a buried urn in a shady place will allow good storage for at least 6 months.

SHAKING METHOD



MIXING METHOD

