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# Rhizobium *inoculants for developing countries*

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A broad account is given of the preparation, use, marketing and distribution of inoculants for leguminous crops.

## INTRODUCTION

LEGUMINOUS crops will play an ever-increasing role as a supplier of protein for millions of people. These valuable plants play a dual role in world agriculture. They not only provide nutritious food, but they increase soil fertility at the same time. With the aid of effective bacteria in nodules on their root systems, leguminous plants have the unique ability to gather large amounts of free air nitrogen. Sometimes these effective rhizobia are present in the soil, but often it is necessary to inoculate the seeds with effective competitive rhizobia prior to sowing to ensure effective nodulation.

The practice of culturing rhizobia in the laboratory to enhance the growth of leguminous crops started almost a century ago, but there were many disappointments and failures during this period. As our knowledge of the intricate relationships between the *Rhizobium* and its host increased, inoculants were improved and gained credibility. This period of development has been described in detail by FRED *et al.* (1932).

## PREPARATION OF INOCULANTS

Inoculants appear inert and lifeless to the naked eye because one cannot see the valuable ingredient, the bacteria. The rhizobia supplied by the inoculum infect root hairs of the host and cause nodules to form. Housed in these nodules, the rhizobia obtain food and energy from their host. The bacteria, in turn, gather or fix atmospheric N for growth of the plant.

### Rhizobium *strain selection*

Different kinds of leguminous plants require different kinds of rhizobia. The appropriate species and strain of bacteria are required for each leguminous host. Plant groups based on infectiveness and on effectiveness have been discussed in detail in a recent publication (BURTON, 1979).

*Rhizobium* strain selection is an important aspect of legume inoculant production. The qualities considered important are: (*1*) Ability to form $N_2$-

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fixing nodules on the plant for which the rhizobium is recommended. (*2*) Competitiveness in nodule formation and survival in the presence of other infective rhizobia. (*3*) Prompt nodulation and good $N_2$ fixation over a wide range of root temperatures. (*4*) Good growth ability in culture medium in the carrier and in soil. (*5*) Persistence or survival in the soil. (*6*) Effective on a wide range of host genotypes. Other important qualities in some areas are tolerance to low and high pH, low phosphorus, high aluminium alkalinity, and high salt concentration.

*Rhizobium* strain selection should be easier now that banks of *Rhizobium* germ plasm already screened for effectiveness are available at a number of research centres. Field testing under local conditions will continue to be needed and the most effective local strains should be included for comparison.

*Culturing rhizobia*

Rhizobia can be grown in bottles, round-bottom flasks, shake flasks, or in any of a wide variety of improvised fermenters. Broth culture with the desired viable cell concentration of 500 to $1000 \times 10^6$ cells/ml can be obtained in a period of 48 to 72 h in a fairly simple vessel. The design of the fermenter should facilitate easy sterilization of the medium, rapid cooling, sampling without risk of contamination, and include a system for aeration with sterile air. Fermenters holding more than 20 litre should be equipped for cooling to avoid heat damage to the growth medium. Cultural media and methods have been given in detail by BURTON (1979) and DATE and ROUGHLEY (1977).

*Inoculant carriers*

The rhizobia are usually mixed with a solid carrier which serves as their home until they are used by the farmer. Peat has been the most commonly used base for commercial inoculants, but it is not available in many developing countries. Some peats do not meet the requirements of a good carrier (GRAHAM *et al.*, 1974).

Carrier materials provide a surface for growth of the bacteria and must permit gas exchange, particularly oxygen. The qualities of a good carrier material are: (*1*) Highly absorptive and easy to process. (*2*) Non-toxic to rhizobia. (*3*) Easy to sterilize. (*4*) Available in adequate amounts. (*5*) Inexpensive. (*6*) Good adhesion to seeds.

Peat, charcoal, clay, bagasse, lignite, coal, compost, straw, and pulverized minerals such as vermiculite and apatite have been used as carriers with varying degrees of success. The advantages and disadvantages of various carrier materials have been reviewed thoroughly in recent publications (DATE and ROUGHLEY, 1977; BURTON, 1979; PACZKOWSKI and BERRYHILL, 1979).

Peat sterilized by gamma irradiation supports a higher population of rhizobia and lengthens the shelf life of the inoculant. The use of imported pre-packed gamma irradiated peat should be considered in areas where no suitable carrier material is available. If suitable peat is a available locally, it can be packaged in high density polypropylene containers, autoclaved and cooled prior to adding the rhizobia.

*Longevity of inoculants*

A peat-base inoculant with 500 to $1000 \times 10^6$ viable rhizobia per gramme should remain effective for four months when the temperature is 30°C or lower. However, the effective life will vary with the form of inoculum and its physiological age. Rhizobia allowed to grow and multiply in the carrier peat survive for longer periods. Rhizobia survival is slightly better in large than in small packages, possibly because of the insulating effect of the carrier peat. Nonetheless, high temperatures should be avoided as much as possible.

Two forms of rhizobial inocula, the polyacrylamide of DOMMERGUES *et al.* (1979) and the lyophilized vegetable oil fertilizer preparation of KREMER *et al.* (1979) are claimed to withstand high temperatures, but further testing will be necessary to establish this quality.

## USE OF INOCULANTS

*Seed inoculants*

The accepted method of using inoculants is to apply an aqueous slurry of the peat base inoculant to seed just before planting. Farmers often mix the inoculant with seed in the drill hopper without using water but this is not reliable (BURTON, 1976). The amount of inoculum applied to seed varies from 2 to 25 g/kg of seed depending upon brand and kind of legume seed.

The number of viable effective rhizobia applied per seed is far more important than the actual weight of inoculum applied because inoculants vary widely in *Rhizobium* content. Fortunately, the '*Rhizobium* per seed' concept is rapidly being adopted in countries where quality of inoculants is monitored.

Most inoculants supply far too few viable rhizobia for maximum benefit, particularly under stress conditions. Inoculation failures are greatly reduced when the inoculum supplies very large numbers of viable rhizobia per seed. In a recent conference on irrigated soya beans in arid and semi-arid lands, HAMDI *et al.* (1979) reported that with $28 \times 10^6$ rhizobia per seed all of the soya bean plants were nodulated, but with smaller numbers failures were common.

The mere application of large numbers of viable rhizobia, however, does not assure success. The form of the inoculum is important. Certain gums and sugars are very beneficial in prolonging viability of rhizobia on leguminous seeds. There is little doubt that very large quantities of effective inocula will be necessary to achieve success under tropical conditions. Standards accepted as satisfactory in temperate climates may lead to disappointment under the severe stresses of dry tropical soils.

*Soil inoculants*

Inoculants designed for application in the seed furrow rather than to the seed have a great advantage under certain conditions. Soils inoculants may be granular or they may be broth cultures or frozen concentrates or rhizobia for use as aqueous suspensions. Also, seed type peat-base inocula may be suspended in water and dripped into the seed furrow. The latter type is being successfully used in Australia (GAULT and BROCKWELL, personal communication).

Soil inoculants are generally used at much higher rates and expressed either as weight or as viable rhizobia per linear metre of row. With the granular

inoculant, the rate ranges from 5 to 30 kg/ha depending upon row width. Liquid or frozen concentrates are usually applied to give equivalent numbers of viable rhizobia where comparisons are to be made with the granular types.

Soil inoculants are generally more expensive because of the larger amount used. However, if suitable carriers are available locally, this type of inoculant should be considered because it has advantages and simple methods of application can be devised for small farmers. When prepared locally, it should not be excessively expensive. When seeds must be chemically protected, seed inoculation may not be possible. Also, the soil inoculant when properly made provides an effective method of supplying the massive number of rhizobia often necessary for effective nodulation, particularly with moisture and temperature stress and where soils are laden with numerous indigenous ineffective rhizobia.

## STANDARDS AND QUALITY CONTROL

In most countries, it is mandatory to put an expiration date on the package (i.e., a date beyond which the product is not considered reliable). The acceptable shelf life will vary with country. It is generally accepted that an inoculant should be used within four to six months after it is made, and that it should be kept as cool as possible. Refrigeration, although ideal, is often not available.

The acceptable standards of viable rhizobia in legume inoculants vary in different countries. Australia requires a minimum of $1 \times 10^8$ viable rhizobia per gramme up to the expiration date. New Zealand's requirement is similar. Canada specifies a minimum of $1 \times 10^6$ viable rhizobia per gramme of inoculum, but the inoculum must provide a minimum of $1 \times 10^3$ viable rhizobia per seed up to the expiration date for small seeds, and $1 \times 10^4$ viable rhizobia for the larger seeds, such as beans or soya beans.

Holland has the highest standard with a minium of 4 to $25 \times 10^9$ viable rhizobia per gramme. Inoculants produced in the United States are evaluated by some of the states by using a so-called 'grow-out' test. The product is applied to surface-sterilized seed at the recommended rate and the seeds are planted in jars containing sterile sand or vermiculite. After five to six weeks, the plants are harvested and examined for nodulation and colour. In Indiana and Ohio, if 90 per cent or more of the plants are nodulated and green, the inoculum is judged satisfactory. The number of viable rhizobia required to pass this test is extremely low.

## MARKETING AND DISTRIBUTION

The information required on the package label varies with country. Countries with the newest regulations generally have the most stringent requirements. In Canada, the inoculant label must show the following: (*1*) Names of legumes for which the inoculum is effective (scientific and common). (*2*) Date beyond which the product should not be used. (*3*) Name of *Rhizobium* species. (*4*) Viable rhizobia per gramme of inoculum or per seed when used according to directions. (*5*) Net weight of inoculum. (*6*) Lot number. (*7*) Directions and rate to use. (*8*) Name and address of manufacturer.

The date of manufacture and the expiration date are required in some countries.

The distribution of legume inoculants is one of the most important aspects to consider. A system which works in one country may not work in another. Since inoculants contain live bacteria, they must be handled as live products if they are to remain effective, and it is very important that all who handle inoculants should realize that the produce is perishable.

In the United States, inoculants are mainly made during the winter and spring seasons just before the leguminous crops are planted. The inoculants are passed from the factory to the regional warehouse. The wholesale seedsman obtains his supply from the regional warehouse. The seed dealer or retailer, in turn, obtains his supply from the wholesale seedsman. The former buys his inoculant along with his seed from the retail merchant. Distribution may require as long as two or three months.

Inoculants must be in the hands of the farmer when he is ready to plant, if they are to be used. The inoculum manufacturer, the wholesaler and the retail seedsman must estimate how much will be needed. If the weather is unsatisfactory or the seed supplies are short, the inoculant may be left in stock after the planting season. Many seedsman are unwilling to take the risk of purchasing inoculants because of these factors. Inoculant manufacturers in the United States have a 'product return system' to reduce the seedsman's risk. Any inoculant left over after the planting season is destroyed and the seedsman receives a credit applicable to purchases the following seasons.

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