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PRINCIPLES OF *Rhizobium* STRAIN SELECTION

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INTRODUCTION

The following account outlines one approach to the selection of *Rhizobium* strains for use in legume seed inoculants. The procedures described were used successfully in a specific program concerned with the selection of appropriate rhizobia for forage legume introductions in acid, infertile soils of tropical Latin America (Halliday, 1979). The principles underlying the approach apply equally well to other programs, and some examples of alternative methodologies are mentioned in the text. Provided they take account of the underlying principles of *Rhizobium* strain selection stressed in this article, individual investigators can modify the techniques and improvise with equipment to suit their own purposes and the facilities available to them.

OBJECTIVE OF STRAIN SELECTION

Strain selection is performed to ensure that a legume seed inoculant contains a strain, or strains, of *Rhizobium* capable of forming fully effective, N_2-fixing nodules on the legume species for which it is recommended and under the conditions of soil and climate in which the legume crop is grown.

CHARACTERISTICS OF IDEOTYPIC STRAINS OF *Rhizobium*

Some characteristics of strains of *Rhizobium* to be used as legume inoculants can be regarded as "essential," whereas others are "desirable" depending on the specific selection objective.

One essential characteristic is the ability to nodulate the legume crop of interest in the field conditions under which it is grown. Such strains are referred to as infective. Strains of Rhizobium which are infective in the field will usually have exhibited competitive ability if they displaced nodulation by native strains present at the site. They will also have been stress tolerant if they successfully nodulated legumes in soils with excesses or deficiencies in their physical and chemical composition.

A second essential characteristic is that the strain be able to fix sufficient N_2 to sustain a level of legume production close to, or surpassing, the production possible if the legume were supplied with nitrogenous fertilizers. Such strains are referred to as effective. Strains which are fully effective are usually carbon efficient and hydrogen efficient as well. The "efficiency" of a Rhizobium is seldom measured during strain selection, and use of the term should be avoided. Effectiveness is usually what is meant.

A third essential character of an ideotypic Rhizobium strain is that it should perform satisfactorily when subjected to the component processes of commercial-scale inoculant-production systems. Inoculant strains must multiply well in bulk culture and be able to mature to high populations in the carrier material, which is usually peat.

A fourth essential character is ability to survive well during distribution to, and use by, farmers. Strains should be tolerant to the anticipated maximum temperature that they will encounter. They must also survive well during the seed or soil inoculation procedures used by farmers. Additionally, they must survive on seed in soil from the time of their application until the emerging legume radicle is susceptible to infection (usually at least 7 days).

Characteristics which are in the "desirable" category are long term persistence and fungicide and insecticide tolerance.

Long term persistence is expected of strains of Rhizobium used to inoculate perennial forages. Implicit in the concept of persistence is saprophytic competence, a summary term for all those traits that permit a Rhizobium strain to live as a stable member of the soil microflora, even in the absence of its legume host. Persistence of strains for annual crop legumes from season to season may be considered a desirable trait in some circumstances as it obviates the need for inoculation in subsequent years. But there may be cropping systems in which carry-over strains from a previous crop may nodulate a following crop relatively ineffectively and even outcompete effective introduced strains. This can occur in rotations of soybean with legumes such as peanut and cowpea that nodulate with the cowpea miscellany of rhizobia.

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Fungicide or insecticide resistance may be a desirable trait when the normal practice is to sow legume seeds pretreated with these substances, some of which are toxic to most strains of Rhizobium.

SCIENTIFIC BASIS FOR STRAIN SELECTION

Rhizobium strains vary widely in the characteristics listed above. Some strains nodulate some genera, or species, or varieties of legumes and not others. This has given rise to the durable, but highly criticized, taxonomy of rhizobia based on their cross-inoculation affinities. Among the strains capable of infecting and nodulating a particular legume, there is great variation in the amount of N_2 they fix; i.e., variation in effectiveness. There is considerable strain variation in the other listed traits as well, and thus an opportunity exists to select superior strains. Unlike higher plants which can be improved through breeding and hybridization, Rhizobium improvement is currently practical only by selection from natural populations.

WHEN IS STRAIN SELECTION JUSTIFIED?

As will be appreciated from the following procedures, the selection of superior Rhizobium strains is a lengthy undertaking. Several years of study may be necessary to complete characterization and testing. Given that strains of Rhizobium for many legumes have already been developed in research laboratories around the world, it makes sense to obtain and use these, rather than to initiate an extensive selection program. Selection of rhizobia is only really justified when the specific selection objective cannot be satisfied by strains held in existing collections. Examples of circumstances under which strain selection may be required are as follows:

a) When the legume of interest is an uncommon species for which there is no recommended inoculant strain.

b) When inoculation of the particular legume with recommended strains of Rhizobium under field conditions fails to give adequate nodulation and N_2 fixation. This can occur if the legume variety is different from that with which the inoculant strain was developed or if the soil and climatic conditions vary from those under which the inoculant was developed.

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SELECTION FOR SOIL STRESS TOLERANCE

This paper describes a step-wise selection procedure for the development of a Rhizobium strain recommendation for legumes planted under a particular soil condition. This approach is unconventional in the sense that strains of Rhizobium in current use as legume seed inoculants are developed for the species of legume with which they will be used, rather than the soil type in which the legume will be grown. In the technologically advanced countries, it is normal farm practice to modify soil conditions to make them suitable for a particular crop. It is not unreasonable, therefore, to expect a rhizobial inoculant for a legume species to perform well wherever that legume is grown. In the developing nations, however, soil amendment is minimal or not practiced at all, and crop plants are often grown under stresses of adverse soil factors that cannot be economically alleviated. It may be unreasonable to expect that a single strain of Rhizobium will perform equally well as an inoculant in the wide array of soil types under which its host legume is grown in the tropics. One reason that legume inoculation is not widely successful in developing countries is that available inoculants obtained from the United States, Australia, or elsewhere do not have strains selected for and adapted to, the extremes of soil stress encountered in the tropics.

There is a widely held view that strain selection and legume inoculation have little potential for improving yields of tropical legumes since tropical legumes are not specific in their Rhizobium strain requirements, and because suitable rhizobia occur universally in tropical soils. There are a few notable exceptions, such as soybeans and leucaena, and thus two categories of tropical legumes were recognized. The promiscuous (P) group can be nodulated by a wide array of strains of tropical rhizobia. The specific (S) group requires specific rhizobial strains for nodulation. The majority of tropical legumes were judged to belong to the P group, and it has been generalized that it is unnecessary to inoculate these legumes with rhizobia, as no benefit would be expected.

The grouping of tropical legumes simply as S or P types is no longer tenable nor useful. Many tropical legumes previously placed in the P group are now known to form fully effective (i.e., high N_2-fixing) symbioses with only a few strains out of the diverse array of rhizobia that can nodulate them. Thus a distinction is drawn between this promiscuous-ineffective (PI) group of legumes and the promiscuous-effective (PE) group (Date and Halliday, 1980). Studies of the Rhizobium affinities of tropical forage legumes, for example, reveal that a majority of them are in the PI group, suggesting a potential for increasing their production by providing appropriate strains of Rhizobium.

The important role played by stress factors in tropical soils

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as modifiers of symbiotic performance is now recognized. Thus, tropical legumes can and do benefit from inoculation when strains are selected specifically for the particular variety of legume being planted and for tolerance of the soil conditions in which that legume is to be grown.

DEFINITION OF THE SPECIFIC SELECTION OBJECTIVE

No strain selection program should be undertaken without clear definition of the specific selection objective(s). The methods of selection employed may need to be modified to suit the objective. The specific selection objective for which the procedures that follow were developed was to select strains of Rhizobium able to nodulate and fix N_2 in association with acid-tolerant legume accessions being introduced to the acid, infertile soils of Latin America.

THE MULTI-STAGE SCREENING APPROACH TO Rhizobium SELECTION

Successful selection of superior rhizobia is favored if the number of strains from which the selection is made is large and diverse. The most meaningful test of Rhizobium performance is field evaluation since this is an integrated appraisal of the various traits that make up a successful inoculant strain. However, the management of field trials to select rhizobia is difficult and costly, even when the number of strains under test is small. Adopting a multi-stage screening procedure that progressively eliminates undesirable strains from an initially high number of contenders to a relatively small number of promising strains for testing at the field level, is one way to reconcile the requirements that selection be from a diverse genetic base, and that strains also be assessed under field conditions.

PRESELECTION OF STRAINS ENTERING THE SCREENING PROCEDURE

It is advisable to include in the screening procedure strains of Rhizobium that originated from a diverse array of host-plant germplasm and that are representative of diverse geographic regions. However, some reduction in the number of strains can be made based on what is known from other selection programs. In general, rhizobia isolated originally from the same genus, and sometimes species, as the legume for which a superior strain is being sought emerge from selection programs as the best strains for use in legume inoculants. Also, when the specific selection objective includes tolerance to a particular soil stress or climatic condition, rhizobia isolated from

legumes growing under those conditions are the most likely to be rated highly in the selection process. Hopefully, there is a Rhizobium collection or collections of authenticated strains of known origin available to the investigator. Otherwise, a collection of strains has to be assembled. Only after checking whether likely strains are available from existing Rhizobium collections, such as the Rhizobium Germplasm Resource at NifTAL, should collection and isolation of new strains be contemplated. Detailed procedures for the collection, isolation, purification, authentication, characterization, and preservation of strains of Rhizobium are described elsewhere (Date and Halliday, 1979; Somasegaran et al., 1979). Preselection of strains with suitable backgrounds should aim to generate a cluster of 50 to 100 rhizobia that will feed into stage I of the strain-selection procedure.

Stage I: Screening for Genetic Compatibility

In this stage, strains of Rhizobium are screened for ability to nodulate the legume of interest. The test used involves a high degree of bacteriological control and is suited to handling large numbers of strains. The system most commonly used is based on growth tubes in which seedlings are raised in a solid nutrient medium under artificial illumination. Seeds must be surface sterilized, usually with concentrated sulfuric acid, hypochlorite, or acidified mercuric chloride. They are pregerminated in inverted, sterile petri dishes containing water agar. When the radicles are 3 to 5 mm long, uniform seedlings are transferred aseptically to tubes containing agar deeps (or slants). Tubes are routinely 2.5 × 25 cm and are capped with a plug of muslin-wrapped cotton wool. Aliquots of 1 ml of suspension of the test strains are added to each tube either at transplanting or 3 to 5 days later. At least three replications of each strain treatment are essential, and five are preferred. Roots of seedlings should be shielded from light and this is best accomplished by using the arrangement in Fig. 1. Alternatively, tubes may be wrapped in Al foil. Two control treatments are required. In one case, the plants are "inoculated" with sterile water only (uninoculated control) and in the other case, they are provided with 70 ppm of N as an ammonium nitrate (or potassium nitrate) solution (plus nitrogen control). Tubes are scored at intervals for the presence or absence of nodules. A word of caution: with many tropical legumes, it is common to observe tumor- or callus-like outgrowths on roots of seedlings raised in growth tubes. These outgrowths occur in the presence or absence of rhizobia and are not nodules. They cannot usually be distinguished from nodules by eye, and it is strongly recommended that plants are harvested from tubes and checked under a binocular microscope for real nodules. "Apparent" nodules lack structural organization and leghemoglobin. Timing of the harvest varies, depending on legume species, but the harvest will usually be about 35 days after sowing.

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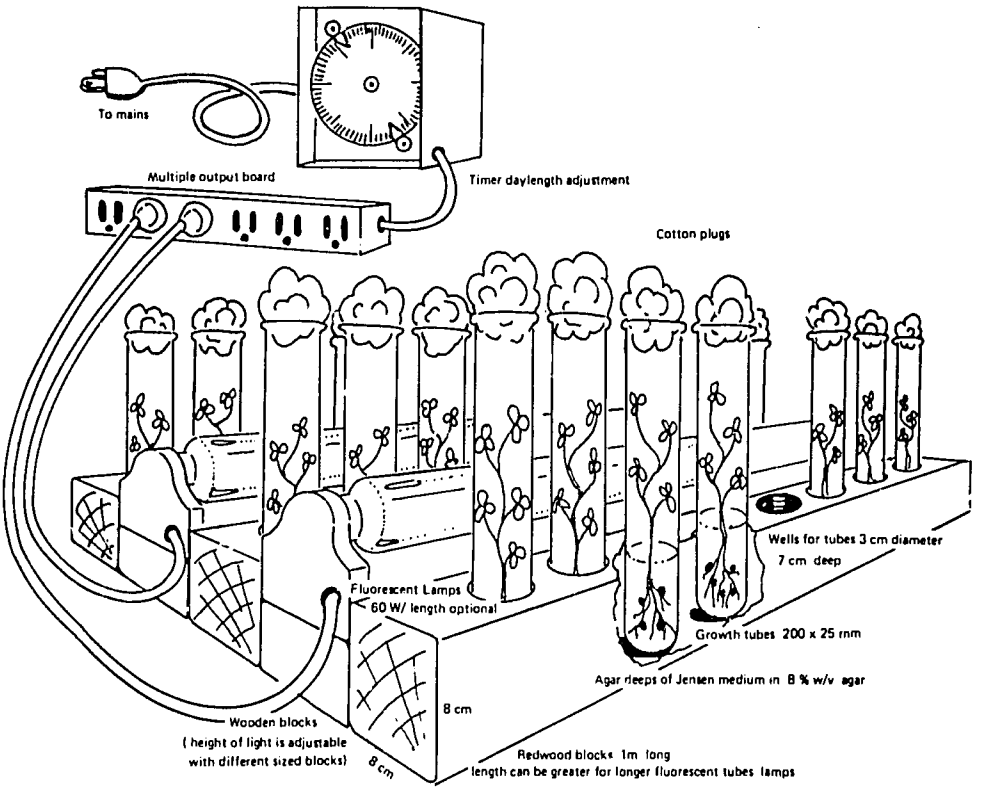

Fig. 1. Low-cost light rack for culturing plants used in plant infection tests.

Some investigators place significance on other data taken on plants grown in growth tubes. "Earliness to nodulation" may be of some value. It is inappropriate, however, to attribute relative N_2 fixation effectiveness to strains based on N accumulation in plants raised under such artificial conditions. The root medium and atmospheric composition within plugged test tubes differ from those which the plants require for optimum performance and may constrain expression of N_2-fixing potential.

Alternate methodologies are required for large-seeded species that quickly become cramped in growth tubes. These include the use of growth pouches or "Gibson" tubes. Growth pouches are made of autoclavable plastic and have an absorbent towel insert. Seedlings germinate in a fold (or are pregerminated and transplanted into the fold) at the upper rim of the pouch. Roots develop within the pouch, where they are nourished by a nutrient medium, and plant tops grow in the open air. The method offers the advantage that effective

nodulation can be reliably determined, but caution in attributing relative effectiveness of strains on the basis of a pouch test is necessary. Modifications of the method include subdividing the pouches with heat bonding to permit a single pouch to be used for several strain treatments, or replications of the same treatment.

The "Gibson" tube contains a long agar slant that reaches to the upper rim of the tube, and the tube is filled to the rim with liquid medium or sterile water. They are capped with Al foil. Radicles of pregerminated seedlings are introduced through a small orifice in the Al. The roots develop inside the tube, and the plant tops grow outside the tube. The method offers similar advantages to those of pouches, namely that effective nodulation shows up readily. Modifications of "Gibson" tubes include omission of the liquid phase or half filling the tubes.

Obviously nodulation in the uninoculated control treatments in stage 1 raises concern about inadequate bacteriological control and invalidates the experiment.

Some texts advocate dedication of entire light rooms for the culture of plants in growth tubes. Most workers will find a low-cost system, such as that in Fig. 1, more than adequate for their needs. The system is highly flexible and can be readily modified to serve for pouches or "Gibson" tubes that require overhead illumination. The issue of light quality has been overplayed, and researchers should not be preoccupied with obtaining special spectrum lamps or with including incandescent lamps in the system. Regular domestic fluorescent lamps have served satisfactorily in the screening procedure described here.

Stage II: Screening for N_2-Fixation Effectiveness

In this stage, the objective is to rank infective strains from stage I in order of potential N_2-fixation effectiveness with the legume species or cultivar of interest. Theoretically, in this test there should be no factors limiting growth of the legume except nitrogen, so that full expression of each strain's N_2-fixation effectiveness is possible. In practice, it is assumed that the nutrient regime and other aspects of growth conditions are not limiting, even though there are known examples of legumes for which standard conditions are not nonlimiting. Sand-jar assembles are used in this test because they permit more realistic growth conditions than tubes, pouches, etc., but they retain the high degree of bacteriological control which is still essential if valid results are to be expected.

The Leonard jar is one example of such a sand-jar assembly and is depicted in Fig. 2. Watering is the most common source of contamination in *Rhizobium* strain testing in pots and in the field.

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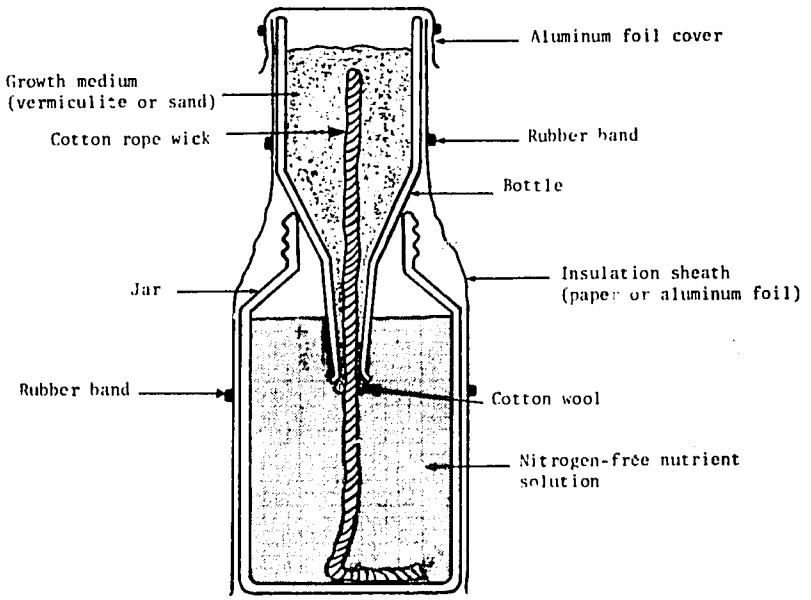

Fig. 2. One example of a Leonard jar assembly. Provided the basic design is followed, the system can be modified to permit use of cheap, locally available materials. The entire assemblies with nutrient solution are autoclaved at 121°C for 1-2 hours, depending on their size. Anticipate some losses (5%) due to breakage of glass components on heating and cooling.

Leonard-type sand jars greatly reduce the frequency of watering and are, therefore, less prone to contamination. Sand jars are easily constructed from locally available materials, but they have the disadvantage that sterilizing them requires a very large autoclave.

As with growth tubes, surface sterilized pregerminated seeds are sown in the sand jars. Four seedlings are allowed to establish and are thinned later to two by snipping off the tops. Drops (standardized rate) of suspensions of strains of Rhizobium are added in the jars 5 days after sowing (one strain per jar). Plants are harvested destructively at a time after sowing that depends on the legume species under test. Usually 60 days after sowing is appropriate.

Date taken on sand jar experiments vary from investigation to investigation and include the following: nodule number, nodule dry weight and/or fresh weight, nodule color, nodule distribution, total plant fresh or dry weight, top weight (fresh or dry), root weight (fresh or dry), acetylene-reduction rate, percentage N in tissues, and total N produced. Of these, total N produced is the most mean-

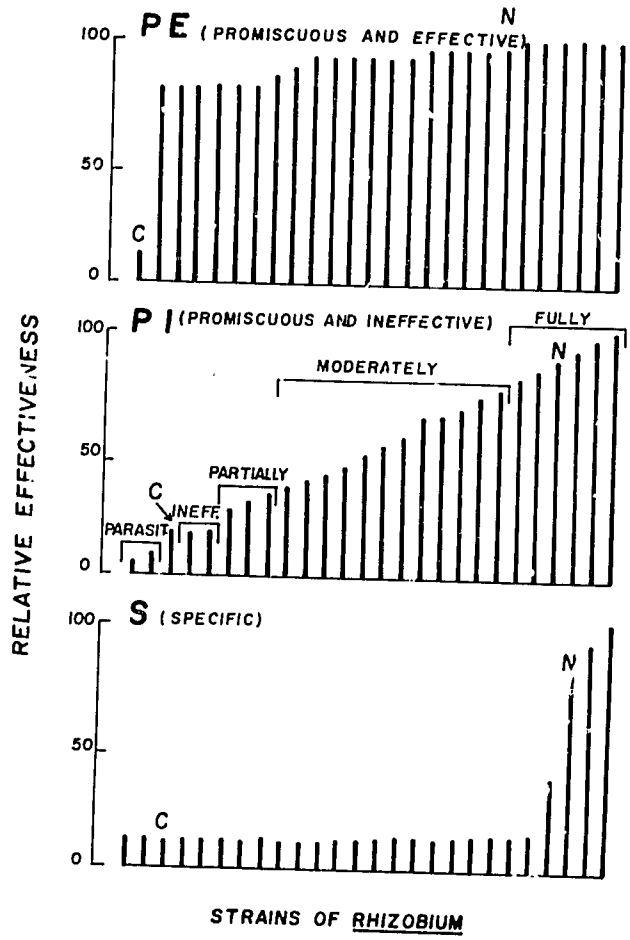

Fig. 3. Hypothetical ranking of strains for potential nitrogen-fixing effectiveness as determined in sand jar assemblies (stage II).

ingful integration of N_2-fixation effectiveness over time, and as this is highly correlated with total plant dry weight, a reliable measure of relative effectiveness of strains of Rhizobium is possible with nothing more sophisticated nor costly than a common balance.

The main problem encountered with this test relates to overheating in the greenhouses or growth rooms where the experiments are performed. Most of the sand-jar trials observed by the author in the tropics are, in fact, selecting high temperature-tolerant rhizobia at the same time! Other problems relate to the occasional failure of the irrigation from beneath, which depends on capillary rise, and breakage of glass components in autoclaving and handling.

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A ranking of strains based on hypothetical yields from stage II trials is presented in Fig. 3. The demarcation of effectiveness categories is somewhat subjective but is nevertheless useful. Strains are assessed relative to the uninoculated control and the N_2 control and described as (in ascending order of merit) parasitic, ineffective, partially effective, moderately effective, or fully effective.

Ordinarily about 30 to 50 strains would be evaluated at stage II in Leonard jars. Three replications are essential, and five are preferred. The top ten strains are chosen for further screening at stage III.

The principal merit of Leonard jar trials are that data on the potential effectiveness of strains of Rhizobium with a particular legume will be upheld in independent screening trials by other investigators. Thus, researchers can exchange information that is stable and demonstrable on the N_2-fixing potential of strains. Pot and field tests, on the other hand, give information of the plant-Rhizobium-soil interaction that may or may not be repeatable at other locations.

Stage III: Screening for Symbiotic Effectiveness under Physical, Chemical, and Biological Stress of Site Soils

The fully effective N_2-fixation effectiveness expressed under stage II conditions will not necessarily be upheld under real field conditions. Thus, before selecting a final cluster of three strains of Rhizobium for field evaluation, it is advisable to subject a larger group (ten) of potentially effective strains to some of the physical, chemical, and biological stresses of soils from the sites for which the inoculant is being developed. This stage is particularly useful if the specific selection objective(s) includes adaptation to a particular stress, such as soil acidity in this case. Stage III still has a value in selection programs, even for "non-stress" soils. In stage II sand-jar evaluation, the test strains did not have to compete against native soil rhizobia.

This third stage involves a pot experiment in which strains are tested with the host plant, and the production is related to that of inoculated control plants and N_2-fertilized plants. Soil is collected from the plow layer and mixed to uniformity to produce a homogeneous experimental material. Unsterilized soil is used. Soil may be amended at fertilizer rates equivalent to field practice, but only the N-control plants receive N (equivalent to 100 kg N/ha). Procedures for calculating the fertilizer additions are detailed elsewhere. Not all soils behave satisfactorily in pot experiments, and other amendments may be necessary, particularly with heavier soils. The following should be considered: (a) sieving to remove large

soil aggregates and stones; (b) addition of high C:N ratio residues such as bagasse at 1 to 2% (dry weight basis) to counter-balance excessive mineralization of N resulting from soil handling; and (c) addition of volcanic cinder, vermiculite, or other materials to improve soil aeration and drainage.

Sowing procedure and inoculation are the same for sand jars in stage II. About 6 to 8 seedlings are planted and thinned to 2 to 4 plants per pot, depending on the species. Thinning is by snipping off the plant tops, rather than by pulling entire plants from the soil. Pot size is optional, but 20 to 25 cm in diameter is usual. Six replications of each treatment are required.

Precautions against cross-contamination in this stage are essential. Watering, which in greenhouses in the tropics is needed daily, is the primary source of contamination. It can be minimized by filling pots so that soil level is 3 cm below the pot rim, watering gently to avoid splashing, using grid or mesh benches instead of solid benches so that pots can drip through onto the floor, raising pots on supports (such as petri dish lids) so that there can be no water flow on the bench surface from the emergent roots from one pot to those of another, and assigning watering to a single, informed individual. Other precautions include avoidance of overheating of the roots and nodules in pots and minimizing nontreatment effects. Pots should be set up in a randomized complete block design but not rerandomized thereafter because of the overriding problem of contamination through handling.

As with sand jars, plant dry-matter production is the most meaningful parameter to be determined and is the basis for ranking strains. The top three strains are promoted to stage IV.

Stage IV: Single-Location Evaluation of Strains of *Rhizobium* and Inoculation Methodology under Field Conditions

Strains emerging from stage III are evaluated for nodulation and N_2 fixation under field conditions. Although the preferred measure of the response by a legume to inoculation with the test strains of *Rhizobium* is grain yield (dry matter production in the case of forages), there are many factors which, under field conditions, can prevent differences in N_2 fixed by the strains being translated into differences in yield. Therefore, field trials should include a mid-season harvest to determine dry-matter production. Plot size should be sufficient to house two fully bordered harvest areas. The standard plot layout used in the International Network of Legume Inoculation Trials (Halliday, 1981a; NifTAL, 1980) is suitable (Fig. 4).

When the specific selection objective includes overcoming soil stress, the field trial at stage IV can amalgamate the strain selec-

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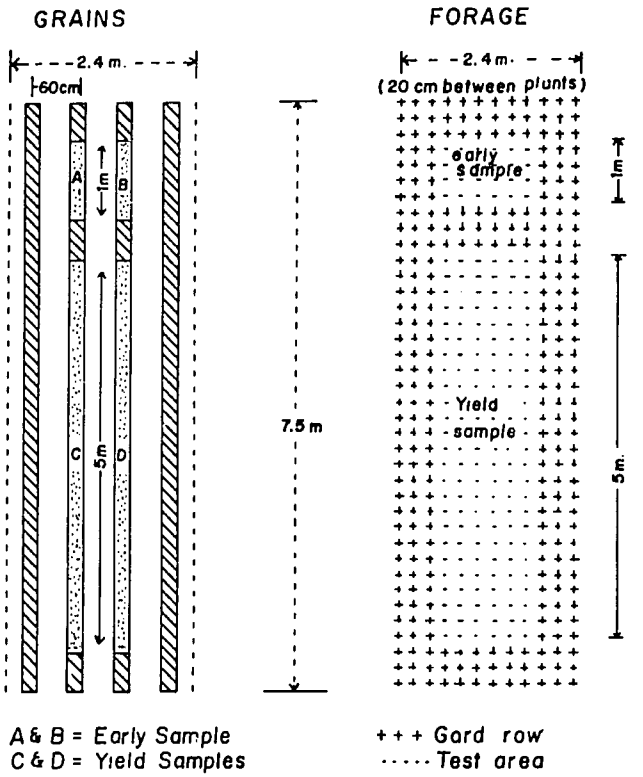

Fig. 4. Plot design for field evaluation of a strain of Rhizobium (stage IV).

tion approach and other strategies for overcoming the stress. In this case, several inoculation methods were appraised for their ability to overcome the effect of acid soil stress on nodulation. Simple seed inoculation with an aqueous suspension of peat-based inoculant containing the test strains was one treatment. Others involved pelleting the inoculated seeds with finely-milled lime or rock phosphate. These treatments were compared to control plots of uninoculated plots and plots fertilized with N. The comparison between these treatments is most valid, in a scientific sense, when there are no other factors limiting plant growth. But the comparison is most realistic when the level of agronomic inputs is economically feasible and similar to that used by farmers in the region where the legume is grown. In the procedure adopted, the scientific validity was considered of lesser importance than the need to be realistic, and a minimal blanket fertilization of elements other than N was applied. Three replications of the treatments were established in a randomized complete block design. Experience has indicated that four replications are desirable.

Precautions against cross contamination are of paramount importance. Common pathways of contamination are careless handling of

inoculated seed at planting time, use of field implements without sterilizing them between plots, tramping from plot to plot (by laborers, animals, visitors, etc.), and run-off and other drainage problems caused by poor site selection.

The best Rhizobium-inoculant method combination is then selected and subjected to further testing in stage V. It could be justified to produce and use legume inoculants based on stage IV evidence, but there remains the risk that the selected strain will be a successful inoculant only in the specific soil and climatic conditions under which it was selected. A further stage (stage V) is essential to determine the range of suitability of inoculants developed for a single location in stage IV.

Stage V: Multi-Location Testing of the Response to Inoculation with Selected Rhizobium Strains

A standardization design developed for the International Network of Legume Inoculation Trials (INLIT) is available for those contemplating multi-location trials on the response of legumes to inoculation with selected strains of Rhizobium (NifTAL, 1980). One of the major constraints to fuller utilization of legume inoculation in the tropics is that there has not been convincing demonstration on a wide scale that yield increases will result with local legume varieties under local soil and climatic conditions (Halliday, 1981a). Stage V trials can assist in deriving the data necessary for predicting more reliably whether a legume will respond to inoculation or not.

The trial has three basic treatments: plants inoculated with Rhizobium, plants not inoculated, and plants not inoculated but fertilized with N.

The comparison between these treatments is most valid, in the scientific sense, where there are no other factors limiting plant growth. The comparison between these treatments is most realistic when the level of agronomic inputs is economically feasible and similar to that used by farmers in the regions where the legume is grown. Therefore, the comparison is made at two fertility levels which, for convenience, shall be referred to as "farm fertility" and "maximal fertility."

Fertilizer levels are determined on the basis of information available locally.

With three treatments at two fertility levels replicated four times, a 24-plot, randomized, complete block design results. The treatments in the first replication can be deliberately arranged to serve as a demonstration in which the treatments that are most frequently compared are located side-by-side to facilitate visual ob-

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servation of treatment differences. Plot arrangement is the same as for stage IV (Fig. 4). Row spacing, planting distance, and seed depend on the legume in question.

The plus N control plots receive 100 kg N/ha but in two doses. At planting, 25 kg N/ha is applied, and 75 kg N/ha is added 4 to 5 weeks later in the case of grain legumes. With forages, the 25 kg/ha is applied at planting, and 25 kg N/ha is applied after each cut (at approximately 3-month intervals).

It is best to sow the "uninoculated" and "N-fertilized" plots first. Only after the seeds in these plots have been covered are the inoculated seeds prepared for sowing in the remaining plots. This minimizes the risk of contamination of the plots that are not to receive rhizobia.

Stage V trials can be used to characterize selected strains for competitive ability and persistence if the inoculant strain is "marked" serologically or with antibiotic resistance. Such strains of Rhizobium can be detected in the nodule population and their ability to compete against strains native to the site determined. These strains can also be detected, if present, in the soil in following seasons, or in the nodule populations of subsequent legume crops sown uninoculated.

The International Network of Legume Inoculation Trials coordinated by the University of Hawaii NifTAL Project is available for 15 agriculturally important tropical legumes. Inoculants developed for INLIT each contain three serologically distinct, effective strains of Rhizobium from diverse geographic and host-germplasm backgrounds (Halliday, 1981a; NifTAL, 1980). Each INLIT is therefore potentially an ecological study of the relative performance of the three exotic strains between themselves and in competition with indigenous soil strains and is also a long-term persistence trial.

ADDITIONAL SCREENING STAGES

For some specific selection objectives, the development of rapid screening procedures may reduce the time taken to develop a reliable inoculant strain, or may greatly increase the likelihood of successful inoculant strains emerging from the step-wise screening previously described. In the case of selection of rhizobia for acid, infertile soils, a laboratory prescreening that preceded the stage I test greatly increased the range and numbers of strains that could be addressed. It eliminated effective strains predestined to fail in the field but which would have passed through stages I, II, and possibly III, consuming time and resources. The prescreening test was based on the reasonable assumption that for a strain of Rhizobium to be a successful inoculant for legumes grown in acid

soils, the ability to multiply well at low pH is an essential trait. Synthetic media were developed that tested the ability to multiply at low pH, and only those strains passing the test were introduced into the step-wise screening program (Date and Halliday, 1979).

Investigators may find it useful to develop such rapid pre-screening steps appropriate to their own specific selection objective(s).

CONCLUDING REMARKS

As with any screening program, there is always the risk that discarded materials that could not be accommodated in the later stages would have performed well in the field. In the procedure described, the stage-to-stage transition that is most problematic is that from stage II to stage III. Rankings of strains in sand jars do not necessarily hold up when subjected to the stresses of site soils. Although 10 fully effective strains are passed across from II to III, examples have occurred in which as few as three of the strains could nodulate at stage III and only one of these was effective (Halliday, 1981b).

When dealing with uncommon legume species, an investigator should be concerned about whether the routine media used in stage I and stage II are, in fact, nonlimiting to growth of the legume plant so that Rhizobium characters can be expressed. As an example of this, it was found that Stylosanthes capitata a legume with high tolerance to soil acidity factors and native only to regions of South America with acid soils, could not be nodulated by any one of more than 100 Stylosanthes isolates (including many specifically from S. capitata) tested in stage I. Nor would S. capitata grow in stage II. Only when the growth medium was acidified to a pH value of lower than 5.0 and the Ca and P levels lowered ten-fold would the plant nodulate and grow.

Even though the screening procedure is lengthy, attempts to shorten the sequence are ill-advised. Recommendation of strains of Rhizobium for legume inoculation without first performing field trials similar to those described in stage IV and stage V would be foolhardy in the face of accumulating data that indicate that site variation in performance of selected strains is common (Halliday, 1980).

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