PD-AAR-689

931021

ISN 41100

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# TRIP REPORT

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Travelled: June 14 to 21, 1980

Itinerary: Washington D.C. to Rome, Italy to Washington, D.C.

Purpose: To participate in the FAO/UNEP meeting on Biological Nitrogen Fixation (BNF) planned to develop a program of FAO activities in BNF. The participants represented many countries. The report and participant list are attached (if not attached, copies may be obtained from L.R. Frederick, DS/AGR/TSWM, (235-8877).

Accomplishments: Recommendations were made for training in inoculant production, more extensive tests, and coordination of ongoing applied BNF programs. To overcome the problems in the use of BNF in developing regions (especially the paucity of data, lack of trained personnel, adaptation of technology). The recommendations aimed at introduction of practical procedures for improving the use of BNF in farming systems, especially with the small-scale farmers. Suggested actions include: (a) obtaining and disseminating results of applied research in both legume production and rhizobia inoculant trials, (b) improved local availability of high quality seeds and inoculants, and (c) special attention to the profitable introduction of legumes into the cropping system. Inoculation of legumes with proper Rhizobium must be combined with other needed production inputs.

Recommendations were made for the following:

I. Inoculant production, handling and distribution:

FAO was encouraged to assist countries in developing high quality inoculant production facilities.

II. Strengthening national capability for legume production and increasing biological nitrogen fixaton:

FAO should build on research knowledge available, develop an extension package of practices and assist in adaptive research and field demonstration programs, and to work with countries to develop a strategy for training and national program support.

III. Training in biological nitrogen fixation:

FAO should cooperate with other organizations and give special attention to training for transmitting validated technology at the farm level.

IV. Coordinating and advisory activities:

FAO needs a full time staff person to give direction and guidance to the BNF program of FAO and to serve as liaison officer with BNF programs in other agencies. A BNF advisory committee that would meet annually was recommended.

During a recent (8/80) vacation in Europe, Frederick visited with Dudal, Matthieu and Roy in FAO/Rome and learned that Mr. Verniau had been hired full-time as associate expert in BNF. Also, regional programs have been developed and submitted to UNDP for consideration. If the FAO program develops, it will affect the AID program in BNF in the following ways.

(1) Provide much needed help to AID activities in legume inoculation trials (the trials needed in some 70 countries with about 20 legumes in several cropping systems will keep many persons active for many years) and with training, workshop and coordination activities.

(2) Increase the urgency for improved BNF technology as more trials are put in place.

(3) Help provide national scientists (may supplement Mission programs) with local assistance in developing and implementing BNF technology into their farming systems.

In short, the work to be done is so large that additional help is urgently and desperately needed. Continuation of AID's relatively modest program is critical to the use of BNF in agricultural development. More effort could be used.

Attachments: Report on the FAO/UNEP Meeting on Biological Nitrogen Fixation (Symbiotic)

Distribution:

DS/DAA/FN, T. Babb
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FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS

UNITED NATIONS ENVIRONMENT
PROGRAMME

# Report

on the

## FAO/UNEP MEETING ON BIOLOGICAL NITROGEN FIXATION (SYMBIOTIC)

FAO Headquarters, Rome, 16 to 20 June 1980

## CONCLUSIONS AND RECOMMENDATIONS

# REPORT OF THE FAO/UNEP MEETING ON BIOLOGICAL NITROGEN FIXATION (BNF)

Rome, 16 - 20 June 1980

## I. INTRODUCTION

The benefits of Biological Nitrogen Fixation (BNF) have been recognized for at least two millennia. Among the ancient Romans, processes that are now recognized as nitrogen fixation were deemed to be important for maintaining soil fertility. Yet it was not until the latter part of the nineteenth century that the role of bacteria (members of the genus Rhizobium) in the nodules of the associated leguminous plants was recognized.

The information obtained at the end of the last century showed that the benefit to soil fertility associated with legumes arises from the capacity of the legume-Rhizobium association to fix nitrogen. Legumes nodulated by Rhizobium active in nitrogen fixation thus became major components of farming systems of Europe, North America and elsewhere, and this biological system was widely used to supply protein to humans and animals and to prevent depletion of soil nitrogen or even add nitrogen to soil. With the availability of inexpensive chemical nitrogenous fertilizers in these countries in later years, the relative importance of many legumes in the agriculture of these areas declined. Nevertheless, the technology remained.

The scarcity or unavailability of chemical fertilizers in many regions, their rising costs in all countries and the frequent paucity of foreign exchange in developing countries have prompted a re-examination of the legume-Rhizobium symbiosis. The capital cost of the introduction of the technology is low, the transportation costs are small and the technology is simple enough to make the inclusion of practices using BNF feasible for farming systems in the developing countries. BNF is not only attractive because it leads to low cost protein and may enrich the soil with nitrogen, but also because it generally gives appreciably less nitrate pollution than chemical nitrogen fertilizers at comparable levels of crop production. Among other environmental advantages, legume BNF technology often contributes to an improved environment by providing vegetation to combat desertification, improve the control of erosion, reduce wind movement of soil and help reclaim degraded land.

An urgent need exists to increase legume production in most developing countries, as grain-legumes are a major component of the protein supply in diets, pasture and forage legumes promote animal production, and leguminous shrubs and trees provide forage and wood for fuel and timber for building. Most nodulated legumes are capable of fully meeting their own nitrogen requirements. This fixation of nitrogen often enhances soil fertility, usually with an immediate, residual benefit to subsequent crop.

This report deals specifically with the advantages of BNF in farming systems making use of the legume-Rhizobium symbiosis, but it should be recognized that BNF also has a role in agricultural systems not involving legumes, such as lowland rice production and some upland tropical grasslands.

Although, as stated above, the requisite technology in the developed countries reached a modest state of advancement in the past and the beneficial results were widely known, problems in the use of BNF exist or remain because of (i) climatic and soil conditions in developing regions, (ii) the introduction of new leguminous crops, (iii) the paucity of data from well-conducted experiments in developing countries to build suitable programmes, (iv) the lack of trained personnel in the developing countries, (v) the absence of facilities to produce Rhizobium inoculants in many areas, (vi) the need for special conditions for inoculant preparations or handling in the tropics, etc. To overcome these problems, certain recommendations are made in regard to training, inoculant preparation, field tests and coordination of on-going applied research. These recommendations are designed to allow for the introduction of practical means for enhancing the role of BNF within farming systems as rapidly and as widespread as possible, with particular reference to small-scale farmers.

The desired end-result of the envisaged activities on legume-Rhizobium symbiotic associations is increased food and fodder production in the developing countries. This aim can only be achieved through a combined set of actions including: (a) dissemination of results from applied research in both legume production and Rhizobia trials; (b) improvement of local availability of good quality seeds and inoculants; and (c) diversification of crop production with special attention to introduction of legumes in the cropping system. Furthermore, efforts to enhance BNF by legumes should be viewed as part of an integrated effort to increase legume production and to establish the role of legumes in the farming system. Inoculation of legumes with Rhizobium is one of a series of inputs to increase legume production; these include new cultivars with high and stable yield potential, amendments for soil fertility, plant protection measures, seed storage methods and marketing facilities.

## II. INOCULANT PRODUCTION, HANDLING AND DISTRIBUTION

The *Rhizobium*-leguminous nitrogen fixing association should be used more effectively in increasing food and forage which are greatly needed in developing countries. Special attention should be directed to the microsymbiont in this association. Full utilization of the legumes depends heavily on development of effective *Rhizobium* inoculants and the establishment of a practical system of distributing them to farmers along with good information on sound practical usage.

### Recommendations

#### 1. Establishment of inoculant production units

FAO should assist countries in the selection of appropriate laboratories in each country that needs inoculants and which does not already have effective production facilities. A qualified microbiologist should be appointed or designated to be in charge of inoculant production in each country. Any essential equipment, material and supplies should be provided. A glasshouse facility is considered essential for testing strains and carrying out a good quality control programme.

#### 2. Selection of strains

Inoculants must be made with the best available strains of *Rhizobia*. These should be highly infective and capable of providing ample nitrogen. They must be able to compete with indigenous strains, multiply in the soil and survive well in the inoculant carrier. Recommended strains can be imported from other laboratories and *Rhizobium* germplasm centres when inoculum production is urgent, but strains should be tested for effectiveness under local conditions as soon as possible. Effective strains can be obtained from MIRCEN Centres, the IARCS (IITA, CIAT, ICRISAT, ICARDA), NifTAL, the USDA Centre. Strains should be isolated from large, healthy nodules which develop on plants from non-inoculated seed and which appear to be providing their host with abundant nitrogen.

The most effective strains should be maintained in a working culture collection and sub-cultures should be deposited at a regional centre such as MIRCENS. All strains must be re-evaluated periodically to ensure that they retain their properties. The most effective strains in the stock culture collection should always be used as standards in evaluating new isolates of *Rhizobium*. Controls in which plants are provided with adequate nitrogen fertilizer should also be included.

#### 3. Preparation of broth culture

The equipment needed to produce broth culture is minimal. Bottles equipped for aeration, shake flasks or simple fermentors may be used. Consideration should be given to equipment already available in the particular laboratory and to the predicted need for inoculants. Some extra production capacity is always advisable. Laboratory equipment useful in *Rhizobium* inoculant production is listed at the end of this chapter.

4. Carrier materials

Peat properly dried and milled has proven to be one of the best carriers. However, it is important to check the suitability of a particular peat and method of drying and processing before it is used in inoculant production. This should be tested with viable Rhizobia because chemical tests are unreliable for complete evaluation of a carrier material. Inoculants prepared from gamma irradiated peat should be employed as standards in evaluating local peats.

In areas without peat, and where domestic peats are being evaluated, peats known to be satisfactory carriers should be obtained from other sources for initiation of inoculant production.

5. Form of inoculant

In the beginning stages the major effort in inoculum production should be directed to seed applied inoculants. In areas with stress conditions, where problems are likely to occur, early attention should be given to preparation of inoculants for soil application, prepared with suitable local carrier materials readily available. Inoculants for soil application may be used to provide larger numbers of Rhizobia and to place the Rhizobia where they can survive until the seeds germinate.

6. Transportation and storage

In BNF programmes, FAO should stress that legume inoculants are live, perishable products and should receive careful handling during transportation and storage. Packages and shipping containers should [illegible]ition against exposure to high temperatures. Inoculants should always be kept in the coolest place available. Inexpensive insulated containers should be used whenever possible.

7. Monitoring of inoculant quality

In its BNF programmes, FAO should stress that quality of inoculants be measured by an independent or officially designated authority. Inoculants should be tested for effectiveness of Rhizobium strains they contain and also for numbers of viable Rhizobia.

Plant tests are essential in determining the correctness of labelling of the inoculants regarding the leguminous seeds on which they are to be used as indicated on the package/label.

As a standard for inoculants we recommend that peat base inoculants contain a minimum of 100 000 000 viable Rhizobia per gramme and that they supply at least 1 000 viable Rhizobia per small seed and 100 000 viable Rhizobia per large seed.

Numbers of viable Rhizobia are usually determined by dilution of the inoculum and applying various dilutions to surface sterilized seed and measuring nodulation after suitable growth periods.

An adequate quality control programme should be carried out in each laboratory which produces inoculants for distribution to farmers. Exchange of inoculants between different laboratories for comparative testing should be encouraged.

8. Inoculant costs

It should be possible to effectively inoculate leguminous plants at a very moderate cost. For example, inoculant for the seed for one hectare of soybeans currently costs around US$1.00. A hectare of well-nodulated soybeans can fix more than 100 kilogrammes of nitrogen.

9. Training and assistance

The training of qualified personnel for inoculant production is critical and should be mandatory. Technicians at existing inoculant production units should be encouraged to attend training courses whenever possible. Any consultancy to assist in developing inoculum production and quality control should be with an adequately staffed agency or company and should be of sufficient duration to enable production techniques to be adequate to ensure high quality inoculants and a good quality control programme. For example, it should span a 3 to 4 month period and provide for continued guidance.

10. Research areas

Research should be promoted on: (i) the selection of local strains of *Rhizobia*, (ii) the technology of inoculant application, (iii) the evaluation of carrier materials, (iv) essential nutrients, (v) characterization of the strains used for inoculant production, (vi) the ability of strains to tolerate local stress conditions, (vii) improved technology on inoculant production, (viii) systems of maturing, (ix) temperature effects on the inoculant in the package and on the seed, and (x) competitiveness.

Laboratory equipment useful in inoculum production:

1. Heat-resistant flasks and bottles with large volume capacity
2. Autoclave
3. Glasshouse or growth chamber
4. Culture room
5. Good binocular microscope
6. Refrigerator
7. Incubator (28°-30°C)
8. Laminar flow hood
9. Hammer mill
10. Sealer for flexible film packages
11. Balances
12. Glassware sterilizing oven
13. Plant drying oven
14. Reciprocal shaker for broth cultures.

## III. STRENGTHENING NATIONAL CAPABILITY FOR LEGUME PRODUCTION AND INCREASING BIOLOGICAL NITROGEN FIXATION

### 1. Information base

1.a. Research conducted by International Agricultural Research Centres, FAO and other International Agencies, and National Research Organizations indicates, for a relatively few agro-ecological situations and crops, the amounts of nitrogen fixed in different systems, and how this can be increased by soil fertility amendments and by inoculation with Rhizobium. There is an urgent need to transmit the results of this research to farmers so that they obtain increased benefit from biological nitrogen fixation. We detail in the following paragraphs the pre-requisites for mounting a successful extension effort for the benefit of the small farmers.

1.b. When legumes such as soybeans are grown in locations where they have not been traditionally grown, there is usually a large response to inoculation. Forage legumes such as some Stylosanthes species, Desmodium spp., Leucaena leucocephala show a similar response.

Responses to inoculation have also been obtained for a range of legumes grown in soils already containing indigenous Rhizobium bacteria capable of nodulating the legume.

In other situations, legumes respond to the addition of nitrogen fertilizer, indicating a deficiency in the biological nitrogen fixing apparatus of that plant in that situation, and the need for research to find the limiting factor(s).

### 2. Development of the extension package of practices for legume production

2.a. Before embarking on any large-scale extension effort, it is essential that information be obtained on the areas where particular legumes are adapted and are likely to be responsive to inoculation, the host cultivar and Rhizobium strains best adapted to these areas, and that a suitable infrastructure be developed for the distribution, application and, in some cases, production of Rhizobium inoculants. The supply as fertilizers of nutrients limiting legume growth also needs to be assured.

2.b. Given FAO's network for assisting National Production Programmes, we believe that FAO is an appropriate agency to adapt to local conditions the existing information on the ways to increase biological nitrogen fixation, to thus serve as the link between research at International Institutes and National Agricultural Research Organizations, and to assist in development of extension programmes promoting the production of legumes and biological nitrogen fixation. These two activities would be bridged by the team of people concerned with legume production.

### 3. Adaptive research and development of field demonstration programmes

3.a. We recommend that this be approached in three phases. The amount of information already existing on the growth of legumes and their response to inoculation differs between countries, regions and crops and will determine

whether phases 1, 2 or 3 as indicated below should be initiated in a particular country. FAO has a role in collating such information. Research is needed to elucidate host cultivar, Rhizobium strain and environment interactions, including the role of the grazing animal, before material can be recommended for inclusion in extension activities. This research should be closely coordinated with any on-going farming systems research as experiments are needed to determine how the legume would best fit in the farming system of that location.

3.b. Phase 1. Adaptive Research Trials. The initial trials are designed to determine whether a particular legume is likely to respond to inoculation and whether there are soil factors limiting symbiotic nitrogen fixation. The minimum design recommended compares treatments with and without inoculation, with a treatment where plants are not limited for nitrogen supply, at two fertility levels - typical farmer fertility level and recommended optimum fertility level for that crop in that region. The design is given in Appendix I and is based on that developed at the Conference on Biological Nitrogen Fixation in the Farming Systems of the Humid Tropics held at IITA in 1975, and detailed at the Workshop on Planning an International Network of Legume Inoculation Trials, held in Hawaii in January 1979. Such a trial should be conducted over several locations and seasons with the legumes of interest to the region. Where responses to inoculation are obtained, these trials should be followed by glasshouse and field experiments comparing (i) methods of inoculation, (ii) Rhizobium strains and their interaction with host cultivars in nitrogen fixation, (iii) soil fertility amendments. This phase of experimentation would take a minimum of three years, and may need to continue for seven years. The trials need to be conducted by a soil microbiologist with appropriate laboratory back-up support.

3.c. Phase 2. Multilocational Trials. The aim of this phase, based upon simple trials, is to (i) measure the effect of inoculation in farmers' fields, (ii) survey the local factors limiting nitrogen fixation, (iii) acquaint extension field staff with Rhizobium inoculation responses before the demonstration Phase 3, (iv) test the host cultivar, Rhizobium strain and soil fertility recommendations developed in Phase 1 at as many sites as possible in the country/region using a simple treatment set of plus and minus inoculation, and compare these with a treatment where enough N fertilizer is supplied to produce maximum crop production, with recommended fertility amendments for the trial site. These trials would need to be conducted over a minimum period of two growing seasons, would be part of a series looking at other aspects of the legume production, and should be conducted and supervised by a field trials officer. Complementary activities during this phase would be the gathering of information on local soil fertility conditions, the farming system(s) and cultural practices followed, on the problems or otherwise of transporting seed, inoculants, fertilizer etc. to the region. It would be desirable to involve existing extension field staff in these activities so that they become familiar with the responses of the legumes in their district.

3.d. Phase 3. Demonstrations. These would be designed to promote and improve legume production in those locations determined in Phase 2 where responses to inoculation and good yields can be obtained. The Rhizobium inoculation would be part of the package including recommendations for cultivar, soil fertility amendments, soil preparations, sowing rates and methods, and plant protection measures. Only when large, visible responses to inoculation can be obtained,

should the demonstration compare inoculated and uninoculated plants. This phase would last a minimum of 2-5 years. Coincident with these demonstrations should be the development of an appropriate system for inoculant production and distribution, improved cultivar seed production, supply and distribution of recommended fertilizer, and provision of plant protection measures.

There would be early indications from the results obtained in Phase 1 of the future role of legumes and the benefits to be obtained by farmers from enhanced levels of biological nitrogen fixation, although it may take at least 10 years from the first adaptive research trials to the stage of acceptance by farmers of any improved package of practices.

## 4. Strategy for training and national programme support

4.a. While the level of FAO involvement in assistance to National Programmes will obviously vary with their state of development, there is a need for sustained involvement by FAO throughout the three phases of activity listed in section 3. Some assistance can be in the form of relatively short consultancies if national capability already exists in particular areas, but for Phase 1 the minimum period of such a consultancy would be 6 months or one growing season, and preferably for 2 years. Assistance required in developing inoculum production and quality control capability is detailed in Chapter II.

4.b. Each legume production team would require a production agronomist who would also assess plant nutrient requirements, a plant protection specialist(s), a soil microbiologist dealing with Rhizobium, and during the extension phase a trials officer experienced in handling Rhizobium inoculation.

4.c. If existing field staff concerned with fertilizer trials are to be involved in legume production extension, they will require specific training and orientation on the role of legumes and biological nitrogen fixation in the farming system and on handling of Rhizobium. Future extension programmes concerned with fertilizer use should also involve a component of legume production and the use of Rhizobium inoculants; again staff will need specific training on the use of legumes in the farming system.

4.d. The soil microbiologist will need access to a laboratory equipped as indicated at the end of Chapter II. A glasshouse with some degree of temperature control is desirable for evaluating Rhizobium strains, particularly their competitive ability in different soils. FAO could assist in acquiring these facilities.

4.e. Most National Research Programmes do not have enough appropriately trained staff to provide the minimum critical mass for our proposed legume production programme to operate effectively. FAO could assist by ensuring that the team is developed and continuity maintained. Training proposals for the soil microbiologists are dealt with in Chapter IV.

4.f. For the programme outlined above to succeed we strongly recommend that a Senior Biological Nitrogen Fixation Specialist with expertise in soil microbiology and legume production be in charge of coordinating and developing FAO's programmes in this area. This specialist would be assisted, where required, by Regional Coordinators. FAO should use its resources at the national level: (i) to ensure that the minimum personnel requirements of the legume production team are met (see 4.b.) reinforcing National Programme capabilities as required with appropriate experts; (ii) to help provide the minimum laboratory and field experiment station facilities.

## IV. TRAINING IN BIOLOGICAL NITROGEN FIXATION

### 1. Justification for training programmes in BNF technology

The reports of those who have surveyed the use of legume inoculation in developing countries have repeatedly emphasized that inadequate numbers of individuals with the essential combination of agronomic and microbiological skills is a serious constraint on the implementation of BNF technology.1/ Despite an increase in the cadre of trained personnel through intensification of training efforts in recent years, particularly by the Porto Alegre and Nairobi MIRCENS and NifTAL, the situation has been alleviated only slightly. Between 1975 and 1979 some 80 participants from 35 developing countries have undertaken training in BNF technology. In some countries however it is still not possible to identify even a single trained individual. In several countries the entire BNF programme is dependent on the interest and competency of just one person. Such a situation is unsatisfactory. Further training programmes are essential to ensure continuity of national research and extension programmes; to equip more individuals with the skills for handling Rhizobia in research and production enterprises; to prepare extensionists in techniques for demonstration of BNF technology to farmers. Trainees should be assisted in integrating into multi-disciplinary legume production teams and provided with information services and essential supply items through follow-up actions.

### 2. Clientele groups

In designing training programmes, it was considered necessary to identify the various categories of potential "trainees". The first group is of key policy and decision makers who control funding and implementation of projects in their institutions. Secondly, there are university and college instructors presently responsible for courses which include a BNF component in their curricula. There are also the applied researchers who adapt technology to conditions in their countries. Another group includes technicians responsible for provision of bacteriological backstopping to legume programmes. Then there are the extensionists who form the front line in delivering BNF technology to farmers.

### 3. Summary of present and anticipated training opportunities

#### 3.a. For policy makers (e.g. directors, commissioners, secretaries of agriculture).

Currently regional newsletters through MIRCENS at Bangkok, Cairo, Nairobi and Porto Alegre and a global bulletin through USAID attempt to meet this specific need. Other newsletters and popular magazines, more general in their content, could carry occasional material giving exposure to BNF (e.g. Ceres, World Bank News, Science, Nature, etc.).

There is an intention to test an approach involving mounting an exposition of BNF technology and its potentials for Peru. This is being undertaken by North Carolina State University (AID-funded) in conjunction with the national agricultural institute. National and provincial agricultural shows can be used to promote an awareness of what BNF has to offer.

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1/ Reports to FAO/UNEP as follows: Africa: Keya, Musa, Obaton, Ayanaba and Agbola; Latin America: Batthyany, Halliday, Freire and Franco; S.E. Asia: Patil.

BNF experts, when travelling in developing countries, should be encouraged to meet with policy makers and alert them to the potential of BNF.

3.b. University and college curricula

Although BNF is covered to a varying degree in university and college curricula throughout the world, more should be done in BNF technology. In addition, there are specialized training short courses as listed at the end of this chapter.

3.c. Degree programmes

Graduate degree programmes in both the developed and developing countries offer the opportunity for advanced specialized training for the researchers clientele group.

3.d. Extension training

For extensionists there are virtually no opportunities for training in the delivery of BNF technology to farmers. The MIRCEN in Porto Alegre and North Carolina State University plan to initiate short courses of this type in Brazil and Peru.

4. Materials and mechanisms for communicating BNF training

Depending on the clientele group training can be imparted by short course intern programmes, seminars, workshops, symposia, conferences, visits, interviews, expositions, agricultural shows and other means. There is a common need for professional quality communication materials to convey meaningfully the principles and use of BNF technology. Specifically the decision makers might be reached through specially developed posters and brochures, well-written light non-technical articles. Where necessary and possible, a list of key bibliographic material, suitable curriculum aids, slide sets, films and a booklet on BNF should be made available to university and college instructors. Practical manuals, such as the IBP Rhizobium Manual and the NifTAL Training Manual, should be made available to technicians. To meet local needs, special consideration should be given to the preparation of appropriate materials such as pocket books, charts and leaflets on BNF for use by extensionists. Special attention needs to be given to the communication of the many features of this technology which is unfamiliar to the farmer.

5. Conclusions

Increased international support has stimulated research and development on BNF technology. However, the impact of such technology has still to be felt at the farm level in many countries. Cognizant of FAO's capabilities in transmitting validated technique at the farm level, FAO should take the lead in the training of manpower required for full implementation of BNF technology.

## 6. Recommendations

It is recommended that:

1. FAO strengthen its existing training programmes in field projects and extension services by including BNF technology.

2. Since no other agency has undertaken to develop a BNF training course and extension materials specifically for extension workers, it is recommended that FAO take necessary action in this regard.

3. FAO should actively sponsor and support training in BNF primarily in the developing countries and in cooperation with other agencies.

4. In order to strengthen the training mechanisms, FAO should make available curricula aids, appropriate audio-visual material, small-scale equipment and the like to national institutions and agencies that provide training in the development and use of BNF technology.

5. FAO, in response to a pressing need, should encourage the regular granting of at least six post-graduate fellowships in BNF (for the regions of Africa, Asia and Latin America) from its various projects, as a strategic means toward generating professional BNF manpower.

## Calendar of Forthcoming Training Courses in Legume-Rhizobium Technology

| Date | Event | Location |
|---|---|---|
| All year | NifTAL Intern Programme | Maui, Hawaii |
| July 1980 | MIRCEN/NifTAL Course | Porto Alegre, Brazil |
| September 1980 | MIRCEN (NifTAL) Course | Cairo, Egypt |
| November 1980 | MIRCEN (NifTAL) Course | Bangkok, Thailand |
| Nov./Dec. 1980 | MARDI/NifTAL Course | Selangor, Malaysia |
| May/June 1981 | C.P. Chapingo/NifTAL Course | Chapingo, Mexico |
| June 1981 | I.N.I.A./N.C.S.U. Seminars | Lima, Peru |
| July 1981 ? | MIRCEN Course | Porto Alegre, Brazil |
| August 1981 | MIRCEN (NifTAL) Course | Nairobi, Kenya |
| 1981 | University of Lyon | France |
| 1981 | SCOPE/UNEP Nitrogen cyclings | Dacca, Bangladesh |
| 1980-1983 | UNEP/UNESCO/ICRO Microbiology Panel Courses | Global ... |
| 1980-1983 | UNESCO Courses | |
| November 1981 | Dept. of Agric. Thailand/NifTAL Course | Bangkok, Thailand |
| 1982 | GRET-INRA-IRAT Course | France |
| 1980-1982 | Courses at the International Agricultural Centres (CIAT, Colombia; ICRISAT, India; ICARDA, Lebanon; IITA, Nigeria) | |

For convenience only the activities taking major promotional and implementation responsibilities are cited. MIRCEN (Microbiological Resources Centre) is an acronym for UNEP/UNESCO/ICRO/host country institutions or laboratories. NifTAL is an acronym for the University of Hawaii/USAID project on nitrogen fixation by tropical agricultural legumes. Each of the listed events is supported to some degree by one or more additional national and international sponsors.

## V. COORDINATING AND ADVISORY ACTIVITIES

Recognizing the importance of biological nitrogen fixation (BNF) for food production in the developing countries and the key role of FAO in promoting BNF in these programmes, it is recommended that FAO take suitable measures to cope with the need for a Biological Nitrogen Fixation Specialist with thorough microbiological training and experience with BNF and legume production. Such a specialist would serve on a full-time basis and should be part of a legume production-farming systems programme at FAO. This individual would give direction and guidance to the BNF programme of FAO in legume inoculant development, field testing, extension and training. He would also serve as liaison officer with BNF programmes of other UN agencies (such as UNEP, UNDP, UNESCO, IAEA).

A great deal of progress has been made by many international, national and private institutions in BNF research to improve legume production. The experience so gained might be used to provide additional experience and guidance to the programme of FAO and to broaden the base of its actions. We therefore suggest that a BNF advisory committee be established. It should attempt to meet annually. The committee would make recommendations to FAO through the BNF specialist on its staff.

In its advisory and harmonizing roles, the committee will serve the equally important function in establishing a medium to:

(a) Identify the gaps in applied research in BNF and to suggest ways and means to fill the gaps.

(b) Stimulate the application through extension of BNF research results already available through transfer of science and technology.

(c) Promote the exchange of information on and harmonization of international meetings, international assistance and training programmes.

(d) Promote the exchange of data and research plans dealing with BNF in developing countries.

(e) Enhance the interchange of micro-organisms and plant germplasm important in the practical use of BNF, and

(f) Maintain contacts with on-going activities in BNF to avoid duplication of effort.

To serve the role of advisors to FAO, promoters of BNF technology and enhancement of information exchange, the committee might be constituted as follows:

- Up to 5 technical individuals (not from UN agencies) to be identified by FAO
- The senior BNF scientists at the International Agricultural Research Centres (CIAT, ICARDA, ICRISAT, IITA, IRRI)
- The directors of the Rhizobium MIRCENS (Kenya, Brazil)
- A BNF scientist from the UNEP/UNESCO/ICRO Panel on Microbiology
- Observers representing UN agencies involved in BNF (FAO, UNEP, UNDP, UNIDO, UNESCO, IAEA) and overseas bilateral assistance programmes with specific commitments to BNF.

APPENDIX I

## *TEST FOR RESPONSE BY TROPICAL LEGUMES TO INOCULATION WITH THE BEST AVAILABLE STRAINS OF* RHIZOBIUM

*Treatments*

There are three *basic treatments*: plants inoculated with *Rhizobium*; plants not inoculated; and plants not inoculated, but supplied with nitrogenous fertilizer.

*Fertility levels*

The comparison between these treatments is most valid, in a scientific sense, when 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 of the three *basic treatments* will be made at two fertility levels which, for convenience, shall be referred to as *farm fertility* and *maximal fertility*.

Fertilizer levels will be developed for specific sites by the Cooperator and the Network Coordinator.

*Plot layout*

Preliminary trials by NifTAL have indicated that four replications are required. The 24 plots are arranged in a randomized, complete block design. It is recommended that all Cooperators adopt the randomization offered on page 17.

The treatments in the first replication are deliberately arranged to serve as a demonstration in which the treatments that are most frequently compared are located side-by-side to facilitate visual observation.

The layout of the plots is described on pages 17 and 18. All of the sampling areas are fully bordered. Row spacing, planting distance, and seed requirement are given on page 18.

*Fertilizing the plots*

Place the weighed quantities of fertilizer (except urea) for each plot in a bucket and mix thoroughly. This is important to achieve good distribution of the compounds such as molybdenum that are used in small quantities. Broadcast the mixed fertilizers evenly over each plot and incorporate into the top 10-15 cm of seed bed. Allow about two weeks before planting. The nitrogen treatments (urea) are applied to the appropriate plots at planting. Follow-up applications of 75 kg N at 4-5 weeks for grain legumes and 25 kg N after each cut for forage legumes are made between rows.

*Planting the experiment*

In this experiment, which has only one inoculation treatment, it is best to sow the "uninoculated" and the "nitrogen fertilized" plots first. Only after the seeds in these plots have been covered over are the inoculated seeds prepared for sowing the remaining plots. This minimizes the risk of contamination of the plots that are not to receive rhizobia. The detailed procedures for inoculation of legume species are given on page 19.

*Data collection and records*

Grain yield and dry matter (DM) of cut forage are the main parameters by which the inoculation response will be measured in grain and forage legumes, respectively. However, there are circumstances in which response to inoculation that is clearly apparent early in the growth cycle may not be translated into a yield response at final harvest due to limiting factors such as disease or drought. Therefore, for grain legumes an early sampling is made at 50% flowering to describe the nodulation in the various treatments and to quantify the response to inoculation (if any) at that stage. For forages the early sample is taken just prior to the first cut (about 3 months after sowing). DM yield from successive cuts is totalled to give annual forage production data. The sampling plan is given on page 20. A format for recording data is given on page 21.

*Supplemental trials*

Some Cooperators may wish to elaborate on this basic experiment to include fertility variables or several varieties of species of interest. Since the rhizobia in the inoculant are characterized serologically, the Cooperator may conduct investigations in subsequent years on the survival and persistence of the strains in the soil.

*Experimental sites*

The ideal site for a legume inoculation trial has:

Very low levels of available soil nitrogen;
No waterlogging or surface water movement problems;
Low populations of effective rhizobia ($<10^3$/g soil);
No history of cropping with legumes; and
No serious acidity or salinity problems.

However, these are seldom the conditions under which legumes are cultivated in the real world. Thus, sites for trials in this Network should be selected on the basis that they are typical of the region in which the legumes are, or will be, grown. Farmers' fields would be an especially appropriate location for these trials. But experimentation in a site with overwhelming disadvantages, such as flooding, would obviously be futile.

*Soil fertility*

The fertility of the soil will be determined by soil testing. Samples representing the total seedbed depth should be taken and analyzed prior to the experiment to aid in determination of the maximal fertility level. If the Cooperator is not familiar with soil sampling techniques, NifTAL personnel can assist with this during the initial site visit or, provide detailed sampling instructions. The sample should be analyzed at a central soil testing laboratory. The following determinations are desirable:

Free nitrate;
Extractable P (Bray P #1 is preferred);
Exchangeable K, Ca, and Mg;
Exchangeable Al and Mn, if soil pH is below 5.2 and 5.6, respectively;
Soil pH (KCl electrode in soil-water slurry);
Organic matter; and
Trace elements, especially if a deficiency is suspected.

*Weed control*

It is important to reduce weed competition and pest damage to a minimum so that these do not obscure the effects of nodulation. Where possible, cultivation to remove weeds is recommended. Otherwise control treatments may be applied as deemed necessary and should be included in this record. However, application of fungicides, etc. (particulary mercury seed coating) at planting is *not* recommended as this may interfere with successful nodulation.

## TREATMENTS AND RANDOMIZATION FOR EXPERIMENT A

*Treatments:*

| Treatment* | Nitrogen | Inoculation |
|---|---|---|
| F-1 | - | - |
| F-2 | + | - |
| F-3 | - | + |
| M-1 | - | - |
| M-2 | + | - |
| M-3 | - | + |

* F indicates farm fertility; M indicates maximal fertility.

*Randomization of treatments:*

| Rep 1 | | Rep 2 | | Rep 3 | | Rep 4 | |
|---|---|---|---|---|---|---|---|
| Plot Number | Treat-ment | Plot Number | Treat-ment | Plot Number | Treat-ment | Plot Number | Treat-ment |
| 1 | F-1 | 7 | F-1 | 13 | F-3 | 19 | M-2 |
| 2 | F-3 | 8 | M-3 | 14 | M-1 | 20 | M-1 |
| 3 | F-2 | 9 | M-1 | 15 | M-2 | 21 | F-3 |
| 4 | M-2 | 10 | F-2 | 16 | F-1 | 22 | F-2 |
| 5 | M-3 | 11 | M-2 | 17 | M-3 | 23 | M-3 |
| 6 | M-1 | 12 | F-3 | 18 | F-2 | 24 | F-1 |

*Plot layout:*

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rep 3 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | Rep 4 |
| Rep 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Rep 2 |

Main approach to experiment

PLANTING INFORMATION FOR VARIOUS SPECIES

| | Between-Row Spacing (cm) | Within-Row Spacing (cm) | Seeds/m of row (approx.) | Plants/m of row after thinning | Plants/ hectare | Seeds needed/ plot | Grams of seed/plot[1] |
|---|---|---|---|---|---|---|---|
| *Arachis hypogaea* | 60 | 5 | 25 | 20 | 333,333 | 750 | 340 |
| *Centrosema pubescens* | 20 | 20 | 10[2] | 5 | 250,000 | 900 | 22 |
| *Cicer arietinum* | 60(30)[3] | 5 | 25 | 20 | 333,333 | 750 | 95 |
| *Desmodium uncinatum* | 20 | 20 | 10[2] | 5 | 250,000 | 900 | 3 |
| *Glycine max* | 60 | 3.7 | 35 | 27 | 450,000 | 1050 | 130 |
| *Lens culinaris* | 30 | 5 | 25 | 20 | 666,666 | 1500 | 63 |
| *Leucaena leucocephala* | 20 | 20 | 10[2] | 5 | 250,000 | 900 | 38 |
| *Medicago sativa* | 20 | 20 | 10[2] | 5 | 250,000 | 900 | 10 |
| *Phaseolus vulgaris* | 60 | 5 | 25 | 20 | 333,333 | 750 | 190 |
| *Stylosanthes guianensis* | 20 | 20 | 10[2] | 5 | 250,000 | 900 | 3 |
| *Vigna radiata* | 60 | 5 | 25 | 20 | 333,333 | 750 | 32 |
| *Vigna unguiculata* | 60 | 5 | 25 | 20 | 333,333 | 750 | 95 |

[1] The seed size of some legume species may vary by a factor of 2 or 3 between varieties. The values given here are for the larger-seeded varieties. If seed supply is a critical factor, then the weight of seed needed should be calculated for the specific batch of seed to be used.

[2] With a 20 cm row spacing, plant 2 seeds per hill and thin to one seed per hill (leucaena will later be thinned further).

[3] When grown as a dry season crop the higher plant population should be used.

## INOCULATING PROCEDURE

This procedure allows the highest possible numbers of rhizobia to be applied to each seed. Recommendations for quantity of gum and inoculant for given weights of seeds are as follows:

| Legume species | Seed weight (g) | Peat-based inoculant (g) | Gum Arabic Solution (ml) |
|---|---|---|---|
| *Arachis hypogaea* | 100 | 10 | 4.0 |
| *Centrosema pubescens* | 20 | 3 | 0.75 |
| *Cicer arietinum* | 100 | 7 | 3.5 |
| *Desmodium uncinatum* | 10 | 4 | 1.5 |
| *Glycine max* | 100 | 10 | 3.0 |
| *Lens culinaris* | 50 | 5 | 2.0 |
| *Leucaena leucocephala* | 50 | 10 | 3.0 |
| *Medicago sativa* | 5 | 4 | 0.4 |
| *Phaseolus vulgaris* | 100 | 8 | 2.5 |
| *Stylosanthes guianensis* | 5 | 2 | 0.2 |
| *Vigna radiata* | 100 | 9 | 3.5 |
| *Vigna unguiculata* | 100 | 8 | 3.5 |

*Preparation of gum Arabic solution*

This operation may take up to four hours and is best performed a day ahead of planting. Heat 100 ml of distilled water in a clean container to near boiling. Regulate the heat to *prevent boiling*.

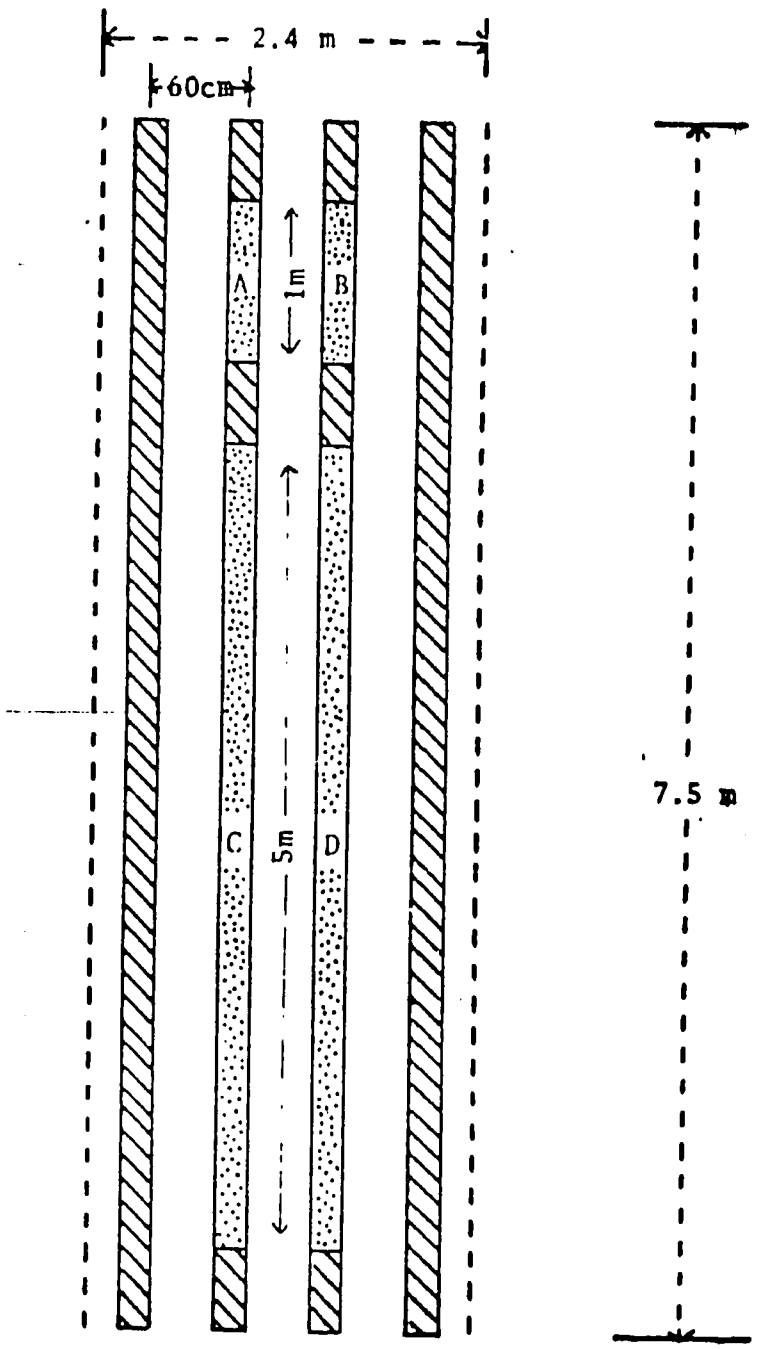
Add gum Arabic powder in small (2 g) lots stirring to dissolve *completely* between additions until a total of 40 g has been added. The resultant solution should be clear and straw-colored. Add the 2.4 g of the precipitated calcium carbonate (provided) to the gum Arabic solution. Stir to evenly disperse the calcium carbonate. Store *refrigerated* until needed.

*Inoculating the seeds*

The procedure is based on inoculation of batches of seed of the weights given in the table above. Place the weighed batch of seed in a polyethylene bag and add the volume of gum indicated in the table.

# PLOT DIMENSIONS AND SAMPLING AREAS

## PULSES

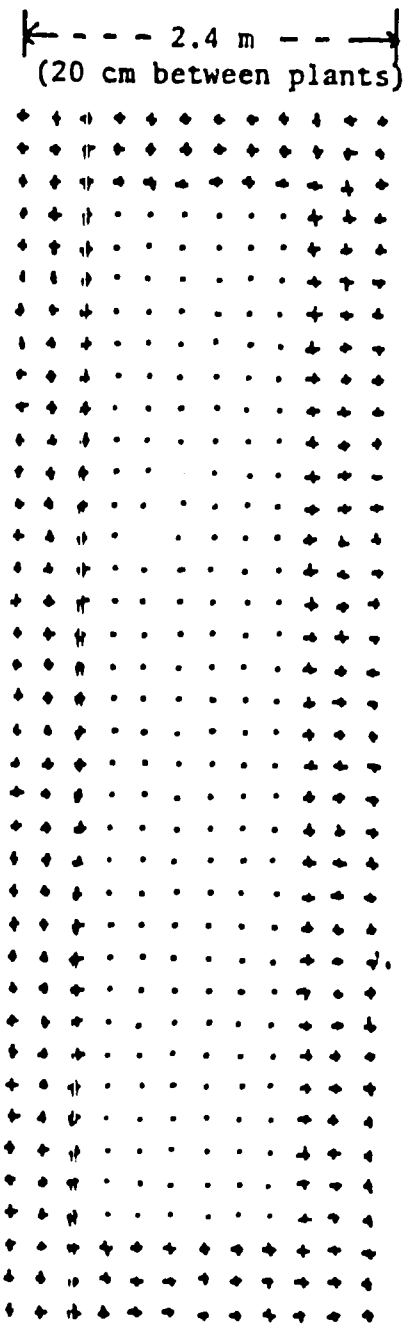



A & B = early sample

C & D = yield determination

The early sample is taken when 50% of the plants are flowering. Ten plants are harvested from A and B. At maturity seed is harvested from C and D.

## FORAGES




+ + + Guard row

. . . Sample area

The early sample corresponds to the first cut. The area sampled can be modified to suit the cutting method. Border should be 50 cm minimum.

Note: When plots are damaged, due to disease or other factors, samples are taken that avoid the affected areas.

## DATA REQUIRED

*The inoculant:*

Viable number rhizobia/g peat at production and at use.
Date of dispatch (if shipped); date of receipt; and date of use.
Highest temperature reached by inoculant during shipping.

*During establishment:*

Comments on germination and stand (with due reference to insect, disease or other problems).
Age of plants when nodules are first apparent--probably between 10 and 20 days after emergence (observations on plants from border rows)

*At thinning:*

Age of plants when (if) plants are thinned.
Nodule abundance, distribution and color (see worksheet 5.2).

*At early sampling[1] (when 50% of plants have flowers):*

Nodule abundance distribution and color.
Total plant dry matter (10 plant/plot sample).
Fresh weight of nodules/plant (store in desiccating vials for serology).
Foliage color: score 1 - 5; 1 = very yellow, 5 = very green.
Chlorophyll, mg/g fresh weight of leaves (optional).
Acetylene reduced, μ mol/plant/h (optional).
Acetylene reduced, μ mol/g dry weight of nodules/h (optional).

*Weather records (or at very least observations):*

Daily max/min air temperature ($^{o}C$).
Daily max/min 5 cm soil temperature ($^{o}C$).
Daily rainfall (mm).
Wind, solar radiation, sunshine hours, pan evaporation (as available).

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[1]*With forages, cuts are made at appropriate intervals (usually each 3 months) and the early sample is taken just prior to the first cut.*

FLOW CHART

Abbreviated for Experiment A
Details of actual requirements given in Appendix 5

Receive inocula and seeds; store in cool place until usage.

Prepare field for planting including non-N fertilizers; approximately two weeks

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Count rhizobia; inoculate and plant seeds; apply N; take soil samples.

↓

Make observations on emergence of nodulation.

↓

Thin as required. (1st set of observations)

↓

50% of plants flowering (2nd set of observations)

↓

Final harvest. (3rd set of observations)

Throughout the growth period, weather records should be kept and weed, peat and disease control requirements and treatments recorded.

APPENDIX II

# FAO/UNEP MEETING ON BIOLOGICAL NITROGEN FIXATION (SYMBIOTIC)

FAO Headquarters, Rome, 16 to 20 June 1980

## PROGRAMME

### Monday, 16 June

#### First Plenary Session

| | | |
|---|---|---|
| 9.00 - 9.30 | - Registration of Participants | |
| 9.30 - 10.45 | - Opening of the Meeting | |
| | - Statement by the Representative of FAO | Dr. D.F.R. Bommer Assistant Director-General, Agriculture Department |
| | - Statement by the Representative of UNEP | O. El-Tayeb |
| | - Statement by the Representative of the FAO/IAEA Joint Division | M. Fried |
| | - "Implications of Biological Nitrogen Fixation on Legume Production in FAO Programmes" | A. Bozzini, FAO |
| | - "Report on FAO activities on Legume Development" | M. Habit, FAO |
| | - "Report on FAO activities on Fertilizer Use Development" | M. Mathieu, FAO |
| 11.00 - 12.30 | - Issue paper: "A Framework for a Coordinated Programme on Biological Nitrogen Fixation" | C.A. Parker and F.A. Skinner |

#### Second Plenary Session

| | | |
|---|---|---|
| 14.00 - 17.00 | - Reports on FAO/UNEP activities on Biological Nitrogen Fixation | C. Batthyany<br>J.J. Drevon<br>M.M. Musa |
| 17.30 | - Cocktail Party offered by Dr. Bommer | |

### Tuesday, 17 June

#### Third Plenary Session

| | | |
|---|---|---|
| 9.00 - 10.45 | - Issue paper: "Some important considerations of *Rhizobium* inoculants for developing countries" | J.C. Burton |
| | - Issue paper: " Activities of the International Research Institutes on Biological Nitrogen Fixation" | A. Ayanaba |
| 11.00 - 12.30 | - Issue paper: "Training Programmes on Biological Nitrogen Fixation" | J. Halliday |
| | - Training Programmes at MIRCEN Centres | S. Keya, J. Jardim Freire |
| | - Training Programmes sponsored by UNESCO | E.J. Da Silva |
| | - Training Programmes in France | M. Obaton |

Fourth Plenary Session

| | | |
|---|---|---|
| 14.00 - 17.00 | - Mission report to Asia and Pacific region | R. Patil |
| | - Activities of MIRCEN Nairobi | S. Keya |
| | - Activities of MIRCEN Porto Alegre | J. Jardim Freire |
| | - Formation of Working Groups on: | |
| | - Inoculant Production, Handling and Distribution | |
| | - Applied Research, Field Demonstrations and Evaluation of Results | |
| | - Training Programmes in Inoculant. Technology and Use | |
| | - Coordinating and Advisory Activities | |

Wednesday, 18 June

First Working Session

9.00 - 12.30 - Work of the four Working Groups

Second Working Session

14.00 - 17.00 - Work of the four Working Groups

Thursday, 19 June

Fifth Plenary Session

9.00 - 12.30 - Work of the four Working Groups and elaboration of preliminary conclusions and recommendations

Sixth Plenary Session

14.00 - 17.00 - Discussion of the preliminary reports of the four Working Groups

Friday, 20 June

Seventh Plenary Session

9.00 - 10.45 - Discussion of the preliminary reports of the four Working Groups

11.00 - 12.30 - Drafting of the final report

Eighth Plenary Session

14.00 - 17.00 - Discussion of the final report

- Other matters

17.00 - Closing of the Meeting

APPENDIX III

FAO/UNEP MEETING ON BIOLOGICAL NITROGEN FIXATION (SYMBIOTIC)

FAO Headquarters, Rome, 16-20 June 1980

LIST OF PARTICIPANTS

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| Dr. M. ALEXANDER | Chairman | Department of Agronomy, Cornell Univers<br>Ithaca, NY 14853, USA |
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| Dr. M. OBATON | INRA - Laboratoire de Recherche sur les Symbiotes des Racines, 9 place Viala Montpellier, France |
| Dr. R. PATIL | Director of Instruction (Agriculture) Agricultural College, Dharwar (Karnataka State), India |
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| Dr. A. BOZZINI | Chief, Crop and Grassland Production Service AGPC, FAO |
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| Dr. J. DE LA VEGA | Technical Officer, Research and Training, Fertilizer and Plant Nutrition Service, FAO |

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| Dr. W. FEISTRITZER | Senior Officer, Seed Production and Certification Unit (AGP), FAO |
| Mr. M. HABIT | Plant Production and Protection Officer Latin America Regional Office (RLAT), Chile |
| Dr. P. HESSE | Regional Coordinator, UNDP/FAO RAS/75/004 Project "Improving Soil Fertility through Organic Recycling" |
| Mr. T. IONESCO | Pasture Improvement Specialist, in charge of EMASAR Programme (AGP), FAO |
| Mr. M. KERNICK | Pasture Improvement Specialist, AGPC, FAO |
| Dr. M. MATHIEU | Chief, Fertilizer and Plant Nutrition Service AGLF, FAO |
| Dr. F. RIVEROS | Senior Officer, Grassland and Pasture Crops Group, AGPC, FAO (not present due to illness) |
| Dr. R.N. ROY | Technical Officer, Research and Training, Fertilizer and Plant Nutrition Service, FAO |
| Mr. TRAN VAN NAO | Forestry Department, FAO |
| Mr. S. VERNIAU | Associate Expert in Biological Nitrogen Fixation (formerly with the French Ministry of Foreign Affairs), AGLF, FAO |
| Dr. C. OFORI | Technical Officer, Soil Management, Soil Resources, Management and Conservation Service, FAO |
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