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PROCEEDINGS: COORDINATING WORKSHOP
BNF LIMITING FACTORS PROGRAM

MAUI, HAWAII, AUGUST, 1985

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TO:

30 June 1986

FROM: S&T/AGR/RNR, Lloyd R. Frederick

SUBJECT: Reprints of Articles with Partial Support of A.I.D. Contracts/Grants

Attached ~~are four (4)~~ is one copy of the following article(s) with the supporting contract/grant number(s):

Roskoski, J.P., L.R. Frederick and G.E. Elkan. 1986
Proceedings: Coordinating Workshop BNF Limiting
Factors Program. University Hawaii

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Attachments: a/s

cc: A. Bertrand
T. Gill

PN-AAY-303

PROCEEDINGS:
COORDINATING WORKSHOP
BNF LIMITING FACTORS PROGRAM
WAILEA, MAUI, HAWAII, USA
AUGUST 15-17, 1985

ORGANIZING COMMITTEE:

Joann P. Roskoski, NifTAL Project
Lloyd R. Frederick, USAID
Gerald P. Elkan, North Carolina State University

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NOT FOR PUBLICATION

These Proceedings are a summary of data discussed at the Conference, some of which are highly preliminary. As a result, materials of these Proceedings are not to be quoted without the permission of the author.

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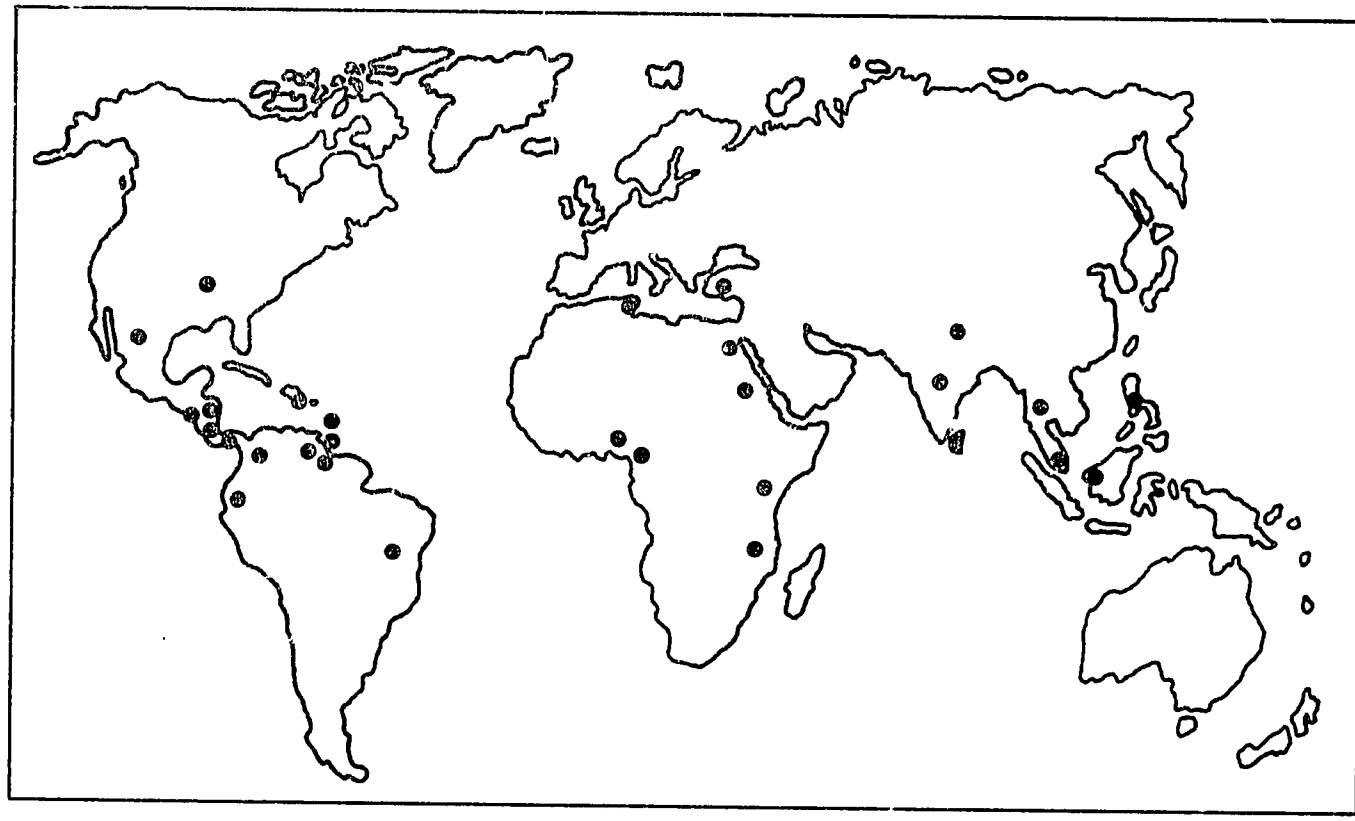
Limiting Factors Grants: A Global Distribution



PREFACE

L. Frederick & G. Elkan

The special grants program for research on factors limiting symbiotic nitrogen fixation for crop production, commonly referred to as the Limiting Factors Program, is now in its tenth year. The program was established to fund teams of U.S. and developing country scientists to collaboratively conduct research on the major factors limiting the full utilization of Biological Nitrogen Fixation (BNF) technology by small farmers in developing countries.

In the MIRCEN News (July 1985), Braun and Verniau (FAO) classify these factors into the following four categories that roughly parallel the Limiting Factors research:

- soil factors: including high levels of acidity, salinity, temperature, drought, nitrate, aluminum and manganese ions;
- life in soils: primarily competition between rhizobial strains and other soil organisms, survival capability and attacks by bacteriophage virus;
- symbiosis partners: including the genetic compatibility between a Rhizobium strain and a host legume cultivar, supply of carbohydrates to the nodules, and plant diseases; and
- man: quality of inoculant, of inoculation techniques, application of pesticides, cropping techniques.

The Limiting Factors Program, which has awarded a total of 68 grants since 1976 with 31 projects currently ongoing, is unique among international research programs for several reasons. First, it links U.S. and foreign scientists into collaborative teams in an "equal partner" relationship which seeks to solve problems in BNF technology that are important to developing nations.

A second unique feature of the program is the exceptionally high quality and quantity of the research. This can be measured by the number of scientific papers published in refereed journals that have resulted from program activities over the past ten years. Lastly, this high level of productivity has been achieved at minimal overhead and administrative cost.

Through the research activities of the Limiting Factors Program, real advances in BNF technology have been achieved. These advances range from the development of oil based-lyophilized rhizobia as an improved inoculant to the backcrossing method of plant breeding that has greatly increased the nitrogen

fixing capacity of dry beans. However, a great deal more research is needed to overcome all the limiting factors in order to provide a BNF technology that will benefit the farmer.

As with other areas of science, many of the simplier questions relating to BNF technology have been answered, leaving the more difficult problems yet to be resolved. For example, indigenous soil rhizobia often occupy many more, if not most, of the nodules even when inoculant is added in large quantities. Many nodulated legumes still show increased yields when fertilizer nitrogen is added, indicating that the BNF system could be improved. Removing these stumbling blocks will require creativity and imagination and perhaps some new approaches, such as molecular biology, to solve old problems.

Answering these research questions is only one step in th process of implementing improved BNF technology on the farm. Successful technology transfer will require collaboration of research scientists and extension personnel, agricultural students, commercial representatives, and farmers. The major responsibility for the task of technology transfer will reside with personnel in each country with U.S. personnel assisting primarily by providing encouragement, expertise, and guidance where requested and appropriate.

For BNF technology to work for the small farmer in developing countries, a complete package of practices must be available. Effective Rhizobium inoculant will make its most significant contribution when placed on good seed of an excellent crop variety, which is then properly fertilized, irrigated, and protected from insects and disease.

Optimizing the benefits of the complete package will require not only the efforts of scientists, but also of educators to help the farmers understand the importance and interrelationship of each component of the package. Proper selection and management of a complete package will result in a profitable crop and economic gain for the farmer and consumer.

The Limiting Factors Program has, from its inception, sought to build an integrated program through the use of planning and coordinating workshops. In the first five years of the project coordinating meetings of principal investigators (P.I.) with the project and program officers were held in conjunction with annual meetings of the American Society of Agronomy and the American Society for Microbiology to conserve time and funds.

In 1980, a grant was made to the University of Hawaii (NifTAL Project) to organize an international workshop that would bring together scientists working on Limiting Factors grants with scientists from other AID-supported projects, the international agricultural research centers, and national research groups working on developing country problems. The workshop was held in March, 1981, and the 726 pages of proceedings were published by CIAT under the title "Biological Nitrogen Fixation Technology for Tropical Agriculture." Participants (178 from 33 coun-

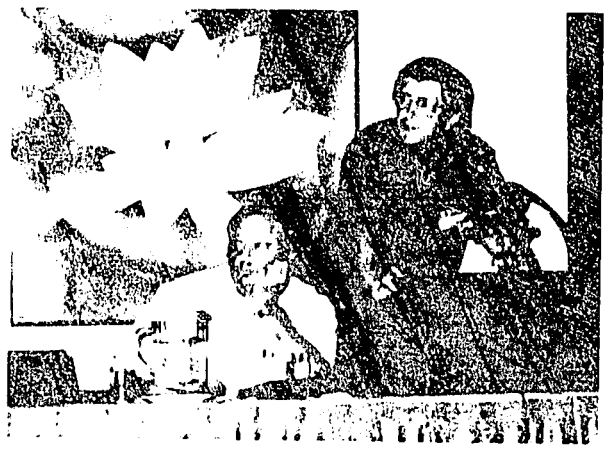
tries) discussed what to do with the current knowledge, and how to work together in its development and application. Cooperative links were developed in seeking technology applicable for tropical agriculture.

The first coordinating meeting specifically for Limiting Factors grantees was held in Washington, D.C., in February, 1982. The meeting's summary report (Appendix 4) identified the most effective and competitive strains of Rhizobium spp. for the major food, forage, and tree legumes and documented the suggested genotypes of legumes to be used as standards for Rhizobium selection. Meetings continued in conjuction with the Agronomy and Microbiology Societies' annual meetings.

The second coordinating meeting specifically for lead investigators of Limiting Factors grants was organized and conducted by University of Hawaii NifTAL Project in August, 1985, in conjunction with the 10th North American Rhizobium Conference at Maui, Hawaii. Both developing country and U.S. collaborators participated for the purposes of exchanging research results, reviewing program objectives and operation, stimulating cooperation, and summarizing program outputs. Highlights of the workshop are given in this report.

We wish to thank the NifTAL Project staff, especially Dr. J. Roskoski, for organizing and managing the workshop. In addition, special thanks go to each of the participating scientists in the program as well as interested observers from CIAT, IRRI, CATIE, USDA, and FAO who attended the workshop and contributed so generously to its success.

(Above) From left: Sabry A. Abdel-Ghaffar, Frank Dazzo

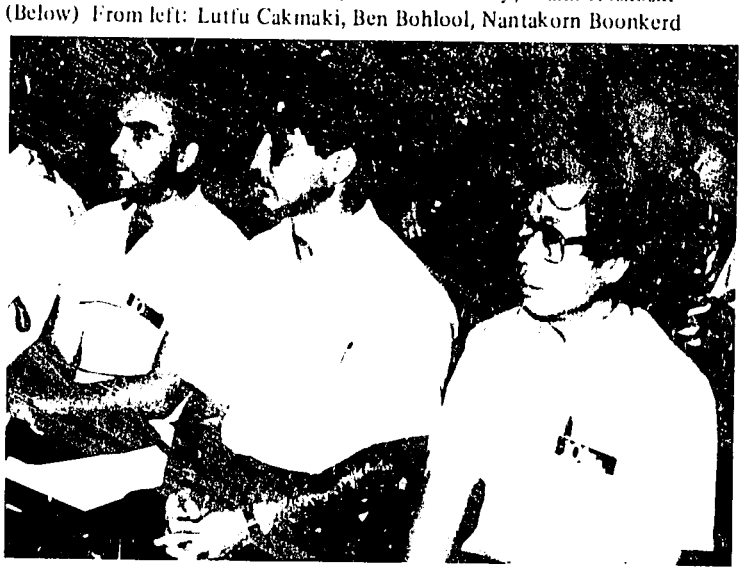


(Above) From left: Gerald Elkan, Peter Graham

(Above) Richard Graham, Remedios Abilay, Joann Roskoski

(Below) From left: Luis Materon, Lloyd Frederick

(Below) From left: Lutfu Cakmaki, Ben Bohlool, Nantakorn Boonkerd



INTRODUCTION

J. P. Roskoski

The Limiting Factors Program coordinational workshop was held in Maui, Hawaii, from August 15 through 17, 1985.

This location and time was chosen by program management to maximize workshop participation and facilitate the attendance of foreign scientists involved in the Limiting Factors Program at the 10th North American Rhizobium Conference which was held at the same location from August 12 through 17.

The workshop was organized and run by the NifTAL Project, University of Hawaii, with funding in the form of a grant from the Limiting Factors Program. Letters of invitation were sent to 60 individuals. These included all current and former grantees. Of the 39 grantees who attended the workshop, 20 were U.S. and 19 were foreign cooperators. In addition to grantees, Dr. G. Elkan, the Limiting Factors Program Manager from North Carolina State University, Dr. L. Frederick, the Program Officer from AID, Dr. C. Smith, the Program Administrator from USDA/CSRS, and interested individuals from CIAT, IITA, CATIE, USDA/Beltsville, and Rhizobium Conference participants brought the total workshop attendance to 60.

Day One of the workshop was dedicated to presentation of project summaries by the Principal Investigators. Presentation lengths ranged from 5 to 20 minutes, depending upon whether a project was new, ongoing, or expired.

On Day Two, the 39 grantees were asked to fill out individual anonymous questionnaires about the goals, structure, operation, management, and value of the Limiting Factors Program. The grantees were also asked to make any suggestions and/or comments about problems they had encountered and ways to improve the program.

After completing the program questionnaires, the 39 grantees were divided into one of four working groups based on the theme of their Limiting Factors grant. Each group was therefore composed of individuals with similar research interests. One member of each group was assigned as chairperson and another as reporter. Each group was asked to answer a series of questions designed to evaluate the scientific output and impact of the research activities supported by the Limiting Factors Program. The questions were centered around the four areas of research supported by the Limiting Factors Program: soil factors that limit BNF, plant factors that limit BNF, rhizobial/inoculant factors that limit BNF, and strain selection/inoculation response.

Day Three of the workshop was devoted to reports from the four working groups, presentation of a discussion paper by Dr. D. Hubbell from the University of Florida entitled "BNF Limiting Factors Program - Past, Present, and Future", brief comments by the USDA Administrator and AID Program Officer, and an open period for comments or suggestions by workshop participants.

These Workshop Proceedings are organized into several sections. The Summary and Recommendations that immediately follow are from the final workshop discussion session. Analysis of the responses of the program questionnaire and the detailed work group reports appear next. Written work group reports were submitted to the workshop coordinator who interpreted these reports into a prose form. These reports were then reviewed and edited by the work group chairpersons. Abstracts of participants' presentations and a list of contributors make up the next section.

The appendices that follow are D. Hubbell's discussion paper (Appendix 1), copies of the various questionnaires used during the workshop (Appendix 2), the participant mailing list (Appendix 3), and the Proceedings of the 1982 Workshop (Appendix 4).

WORKSHOP SUMMARY AND RECOMMENDATIONS

PROGRAM IMPACTS

The overall concensus of workshop participants was that the Limiting Factors Program had been extremely valuable to them--both professionally and personally. They noted that their ability to do research and understand the problems of developing nations had increased, and that their institutions had benefitted not only from the acquisition of the material and tools necessary for scientific research, but also from enhanced contact with other international programs.

Limiting Factor Program grantees strongly asserted that the collaborative mode of linking U.S. and foreign scientists was highly successful in terms of the quality and quantity of scientific research accomplished and of benefits gained by both members of the collaboration. However, they felt that although advances had been made in understanding and eliminating some factors that limit BNF in developing country agriculture, much remained to be done.

Workshop participants were unanimous in their support of the Limiting Factors Program and expressed concern that budgetary constraints might reduce its activities at the very time when policy makers in developing countries were becoming convinced, in many cases through the activities of Limiting Factors grantees, that BNF is a biotechnology of tremendous potential benefit for their nations. Foreign cooperators, however, also acknowledged that responsibility for the actual promotion and implementation of BNF in their countries resided with them.

Because participants felt that the program had been not only highly successful in achieving its goals but had done so in an extremely cost effective manner, they urged that the Limiting Factors Program continue and, if possible, find additional support to increase its activities.

REAL WORLD IMPACTS

Grantees were asked if practical spin-offs had resulted from their Limiting Factors grants. Despite the fact that the Limiting Factors Program is research oriented, several cooperators reported real world impacts as a result of Limiting Factors grant activities.

- In Egypt, a local seed company has incorporated inoculation as a standard management practice after seeing the inoculation response obtained on chick peas and *Vicia faba*.
- In Brazil and Venezuela, rhizobial strains used in inoculants were changed as a result of work that selected better strains.
- In Honduras and Brazil, high nitrogen-fixing bean cultivars

are being produced on demonstration farms.

- In Latin America, oil based-lyophilized culture inoculants are being evaluated as part of a UNDP-funded program.

- In Thailand and Malaysia, legume inoculant production centers are dependent on Limiting Factors grantees.

GRANTEE SUGGESTIONS

A major feature of the workshop was an assessment of the Limiting Factors Program. Grantees contributed specific suggestions and/or comments about program goals, design, implementation, operational framework, or management by means of an anonymous questionnaire. Respondents were half non-U.S. (19) and half U.S. (20) collaborators. Two-thirds had not collaborated with their co-PI previously.

About 80% of the foreign scientists whose grants had expired were continuing collaboration with their U.S. co-PIs. Twenty-five percent indicated serious problems, such as lack of vehicles, lack of money, and USAID and host country red tape. Eighty percent of the foreign grantees felt they had contibuted significantly to the project design. Seventy-two percent of all grantees acquired new skills--ranging from new understanding of developing nations to new scientific techniques. Eighty percent felt their institutions had benefitted from the collaboration. The greatest personal benefits included experience, professional contacts, ideas, exchange of technology, and appreciation of international problems.

Overwhelming support was given to collaborative activities like the Limiting Factors Program because collaborators (1) obtain training and experience, (2) share ideas and resources to accomplish a common task, and (3) gain better international understanding.

A summary of specific comments follows:

1. Goals/focus of the Limiting Factors Research program should emphasize better nitrogen-fixing, higher yielding cultivars, include more genotypes and rhizobial strains, and increase emphasis on biotechnology and research findings implemented in farmers' fields through extension-oriented activities.

2. Training was desired with greater opportunity for non-U.S. scientists to work in the U.S. cooperator's lab either as a visiting scientist or as a graduate student.

3. Communications always need improvement even though workshops are helpful. Requested was a list of current projects and grantees to encourage collaboration, an annual publication of brief reports for all grantees, and more workshops.

4. Funding, as expected, should be increased with greater flexibility and reduced paperwork. More advanced countries should fund their own share of cooperative projects.

5. Management suggestions included

expanding the decision-making group, eliminating grantees involved in other AID-supported programs, getting more U.S. universities involved, offering greater flexibility to accommodate political changes, supplying simple equipment to overseas grantees, and reducing paperwork, such as memoranda of understanding.

SIGNIFICANT RESEARCH RESULTS

Research reported came from projects of different maturity ranging from those newly initiated to those completed. Author abstracts of the research reports are presented in a later section.

Some highlights from the research reports are as follows:

Improved Germplasm For Nitrogen Fixation

- Using local beans as a recurrent parent crossed with high nitrogen fixing genotypes, several inbred backcross lines had better nodulation and higher yield than local cultivars (EAP, Honduras and U. Wisconsin).

- Peanut genotypes are being screened for shade tolerence and nitrogen fixation. Shade tolerant genotypes are needed for greater productivity when intercropped, especially in agroforestry (UPLB, Philippines and N. Carolina State U.).

- Cowpea and mungbean have significant genetic diversity in nitrogen fixation capability. Heritability was also influenced by the rhizobial strain used. With cowpea, rhizobial inoculation significantly increased seed yield. The increase was equivalent to that obtained with 100 kg fertilizer nitrogen per acre (Sri Lanka and Texas A&M U.).

- Eleven of 126 bean cultivars were tolerant of low pH when dependent on symbiotic nitrogen fixation for growth. Tolerance of bean and tolerance of rhizobia were not always directly related (INIAP, Ecuador and U. Minnesota).

Crop Responses to Rhizobial Inoculation of Legumes

- Peanuts planted in Cameroon were inoculated in the row with a liquid peat slurry. Up to a 26% yield increase was noted when peanut 280206 was inoculated with *Rhizobium* NC92. In further tests, some peanut by *Rhizobium* combinations yielded as well as those fertilized with 196 kg urea/ha, indicating that BNF can produce optimum yields (IRA, Cameroon and N. Carolina State U.).

- *Leucaena leucocephala* and *Acacia pennatula* were significantly taller when inoculated with a selected strain of rhizobia at a responsive location. Tropical trees can benefit from inoculation of nursery stock with rhizobia (INIREB, Mexico and U. Arizona).

Soil Factors Limiting Nitrogen Fixation

- Phosphorus fertilizer increased nitrogen fixation and seed yield with fababeans (*Vicia faba*), up to about 100 kg P_2O_5/ha but levels of more than 140 kg P_2O_5/ha decreased nitrogen fixation and seed yield (Alexandria U., Egypt and Montana State U.).

- Studies of interactions between nematodes and legume genotypes indicated that the three Meloidogyne species studied had little or no direct effect on nodulation or nodule activity. Reduced nitrogen fixation in the presence of the nematode invaders is due to plant breakdown (Dept. Agr., Malawi and U. Florida).

- Factors limiting bean nodulation and yield in the northcentral plateau region of Mexico are (1) lack of indigenous bean rhizobia and (2) phosphorus fertility. These can be overcome by proper rhizobial inoculation and phosphorus fertilization at 40 Kg P_2O_5/ha (CENMAR, Mexico and New Mexico State U.).

Higher Quality Rhizobia Inoculants

- Rhizobium inoculant is perishable, but farmers can protect it in a container buried in the ground. A unit placed in the shade and 25 cm below ground had a stabilized temperature of 25.5 degrees C even though ambient air temperature reached 36.7 degrees C. Inoculant storage life is being studied (U. Costa Rica and the Nitragin Co., WI).

- Stability of the nitrogen fixing characteristics of rhizobial strains is important in inoculant production. To minimize instability, rhizobial cultures should be increased in mass, not from simple colonies. Also, screening for enhanced survival under simulated field conditions can provide strains that are well adapted (Dept. Agr., Thailand, Min. Agr., Guyana, and Texas A&M U.).

DETAILED INFORMATION

(Above) From left: Carlos Ramirez, Juan Carlos Rosas, Blanca de Hernandez

(Above) Joann Roskoski, Caio Vidor

(Above) From left: Richard Weaver, Deane Weber

(Below) Eduardo Schroder, Consuelo Estevez

(Below) From left: Joe C. Burton, Chris vanKessell

DETAILED SUMMARY: LIMITING FACTORS PROGRAM QUESTIONNAIRE

The following information is a summary of the answers given on the Limiting Factors Program questionnaire.

PROFILE OF GRANTEES

Half the respondents (19) were foreign and half (20) U.S. collaborators. Grants were equally split between expired (11), ongoing (15), and newly approved (12). For half the respondents, it was their first overseas project (17 vs. 18) and the first time they had collaborated with a U.S. scientist (14 yes vs. 12 no). Of those foreign collaborators who had previously worked with U.S. scientists, two-thirds had not worked with their Limiting Factors co-PI before. Of those who had worked with their co-PI, the time intervals varied about equally from 1 to 4 years.

Two-thirds of the foreign collaborators wrote part of their original project proposal and conferred several times with their U.S. collaborators during proposal preparation. 80% of the grantees felt that they had contributed significantly to the project design. Collaborators felt that co-PIs accomplished their part of the work plan and 32 out of 33 respondents reported no personality incompatibilities.

A fact of major significance is that 82% of the foreign scientists whose Limiting Factors grants had expired, still collaborate with their U.S. co-PI.

COMMUNICATION

Communication problems, in general, were nonexistent (15 of 38) or occassional (21 of 38). Only two grantees reported severe communication problems.

Grantees felt that improved communication might be achieved through better telephone and perhaps computer linkages; an initial meeting between U.S. and foreign co- PIs; attempts to improve langauge skills; less government red tape; and simpler formats for paperwork.

FINANCES

With respect to financial considerations, timely disbursement of funds did not seem to be a problem and the majority of respondents felt that funds were fairly budgeted for the tasks outlined in the project proposal (29 of 33). The solutions to money problems suggested by the 4 respondents who did not feel that financing was fairly distributed included the following: more money for travel, better supply sources overseas, calculation of inflation rates into budgeting, and overall better cost estimates.

MANAGEMENT & SITE VISITS

Respondents were equally divided about the usefulness of program management in solving problems. Those grantees that had been helped by management had been helped with budgeting, travel arrangements, coordinating with local USAID officials, starting dates, the early planning trip, cutting through University red tape, selecting strains, and purchasing

equipment. About half the projects had problems getting the memorandum of understanding (MOU) signed which delayed the start-up of the project. However, 74% of the respondents felt there was no problem in coordinating overall grant activities.

An overwhelming number of the respondents noted that the number of visits by their co-PIs and the length of visits were adequate. The most serious problems the PIs experienced were lack of vehicles (5 grantees); lack of money (5); USAID and U.S. red tape (4); communications (1); transfer of money (1); delay of funding (1); sending supplies overseas (1); U.S. immigration (1); foreign politics (1); and transportation overseas (1).

COLLABORATOR BENEFITS

Twenty-one of 29 respondents said they acquired new skills as a result of participating in the program. These skills/knowledge included the following: understanding problems and restrictions in developing nations, learning new scientific techniques, acquiring communication skills, and dealing with U.S. red tape. In addition, 80% thought their institutions had benefitted from their collaboration in the program through enhanced exchange with other programs, training, acquisitioning equipment and supplies, sharing resources, gaining new knowledge about BNF, and gaining scientific expertise.

The greatest personal benefit that PIs said they obtained from participating in the program were: graduate degrees (2 grantees); publications (2); experience (10); knowledge of LDC problems (3); appreciation of international problems (5); exchange of technology (5); institution building (3); professional contacts (7); exchange of ideas (5); equipment (1); and project completion (1).

Respondents were overwhelming in their support for collaborative activities of the type exemplified by the Limiting Factors Program. The reasons they cited were as follows: the chance to obtain training and experience; better international relations; sharing resources to accomplish a common task; better understanding of problems in developing countries; the opportunity to exchange ideas and attack similar problems. The only negative response on the value of collaborative programs cited politics as the reason.

Respondents were almost equally split on whether there had been enough coordinational meetings. About 68% of the collaborators had been visited by either the Limiting Factors Program manager, a USDA official, or the AID project officer during their grants. Of those who had been visited, 73% found the visit useful and 27% found the visit counterproductive. Of those who had not been visited, 63% would have welcomed a visit.

SUGGESTIONS

Finally, each respondent was asked to contribute suggestions and/or comments about the management design, implementation, operational framework, or goals of the Limiting Factors program. The suggestions received--with the number who had cited them in parentheses--follow.

ON GOALS / FOCUS OF THE PROGRAM

1. Breeding better nitrogen-fixing, higher yielding cultivars should be emphasized (2).

2. The objective of the program should be to make inoculation work for the farmers (1).

3. More strains and genotypes should be included (1).

4. Emphasis should be placed on biotechnology (1).

5. Research findings should be implemented in farmers' fields (1).

6. More emphasis should be placed on extension-oriented activities (4).

ON TRAINING

1. The program should be more ambitious in its training activities (1).

2. The non-U.S. scientist should have the option of working in the U.S. cooperator's lab for a period of 3-4 months (1).

3. Students from developing countries should be used more in the projects (1).

ON IMPROVING COMMUNICATIONS

1. A list of those currently funded by the program and a summary of their research projects should be made available to all grantees to encourage collaboration and avoid duplication (3).

2. Abstracts of all projects should be published and distributed annually to all grantees (1).

3. Workshop reports should be circulated (1).

4. More meetings of the type held in Hawaii should be planned (1).

ON FUNDING

1. More money is needed (3).

2. Brazil, Thailand, and Mexico have resources to fund their own projects (1).

3. There should be more flexibility in funding (3).

4. Funds should be sent directly to cooperator's institution and not to coordinator's agency when the two are different (1).

5. The amount of red tape involved in the transfer of funds should be reduced (1).

ON MANAGEMENT

1. The program has too few management people making all the decisions. There should be more input from others (2).

2. BOSTID grantees should be eliminated from consideration in the Limiting Factors Program (1).

3. More U.S. universities should be involved in the program (1).

4. There should be more programatic flexibity, so that after a project is started its goals can be changed if political changes within the developing nation require it (1).

5. Simple equipment should be sent overseas (1).

6. The program design should be improved (1).

7. The number of reports required by the program should be reduced (1).

8. Do away with the memorandum of understanding (1).

9. There should be a better integration of Limiting Factors and CRSP projects (1).

REPORT OF WORK GROUP 1 ON PLANT FACTORS THAT LIMIT BNF

Work Group 1 Members:

J.C. Miller, Jr., Chairperson
J.C. Rosas, Rapporteur

J. Wynne
G. Fernandez
R. Abilay
V. Saka
S. Zuluaga
D. Pradham
D. Baltensperger

LIMITING PLANT FACTORS

The first task of the group was to select one of four plant factors that we felt was the most important in limiting BNF (refer to Questionnaire for Work Group 1 in Appendix 2 for specific questions and possible answers, for each topic discussed in this report).

We decided that the production potential of the plant and the genetic capability of the plant-rhizobia system for BNF were interlinked factors that taken together were the single most important plant factor limiting BNF. However, any factor which affects plant growth will undoubtedly affect BNF. Not surprisingly, the genetic capability of the plant for BNF was also the most common limiting plant factor studied by members of this work group in their Limiting Factors grants.

Beans, cowpeas, peanuts, and pigeon peas were the four legume crops in which we felt this limiting factor was most important. These species were selected because they are crops of major importance whose BNF systems have, to date, received little attention from plant breeders.

While some molecular biology techniques might be useful for eliminating plant factors that limit BNF, the consensus of the group was that, at present, such techniques are not available--and even when they are--they may be difficult to apply to improve a quantitative trait like biological nitrogen fixation.

Similarly, the group felt that while the development of promiscuous nodulating plant lines may hold little promise for eliminating the major plant constraints to improved BNF, a preferred approach is to develop lines that support nitrogen fixation whether it be with introduced or indigenous rhizobia.

With respect to the use of the lists of plant cultivars and rhizobial strains recommended as standards by the 1982 coordinational workshop, most members of the work group with new grants were unaware of the existence of the lists while most other members had

used the recommended plant cultivars in their Limiting Factors grants research programs.

With respect to the recommended rhizobial strains, work group members with both old and new grants were not sure if the rhizobia they used in their studies were recommended strains. Apparently, the lists had not been disseminated to those who had not attended the 1982 workshop.

RESEARCH STRATEGY

The group was in general agreement that plant improvement and removal of plant limiting factors held the greatest potential for increasing BNF and that plant limiting factors had not received sufficient attention in the past. We therefore recommend that more grants of longer duration focused on eliminating the plant constraints to increased BNF be awarded.

We urge that these grants involve on-site evaluation; require a team approach (ideally a rhizobiologist and a breeder or agronomist); and contain training of local manpower so that local genetic material can be evaluated and improved. Research efforts should focus on a few crops and people. These efforts should have funding levels increased and be allocated to a few large, long-term projects rather than many small, short-term ones.

If the strategy above was adopted and successful in eliminating the plant constraints to improved BNF, then the group felt the next most important factor to overcome was environmental stresses, in particular, drought and soil factors.

FUTURE DIRECTIONS

The consensus of the group was that if one of the five topics currently funded by the Limiting Factors program had to be eliminated it should be strain testing and inoculation response studies. The group felt that many good strains for most legume species already exist and that more energy should be expended on implementing inoculant technology and the use of inoculants. The four remaining topic areas ranked in order of importance were:

- Plant factors that limit BNF
- Constraints to implementation of inoculant technology
- Soil factors that limit BNF
- Rhizobial factors that limit BNF

If, it were necessary to cut all but one of the program areas, we concluded that the area that should be retained is plant factors that limit BNF with the recommendations that work should be funded on a few major crops and--given the interrelationship between the plant and microbe in the nitrogen fixing symbiosis--an interdisciplinary approach should be encouraged.

We concluded that the overall goal of the Limiting Factors Program should be to increase legume productivity without the use of nitrogen fertilizer. Finally, if an additional topic area could be added to the Limiting Factors Program, it should be demonstration of the benefit of BNF to farmers using on-farm research and extension.

REPORT OF WORK GROUP II ON STRAIN SELECTION AND INOCULATION RESPONSE

Work Group II Members:

D. Bezdicek, Chairperson
I. Pepper, Rapporteur

E. Pardo
R. Graham
C. van Kessel
J. Elkan
D. Johnson
A. Sexstone
Z. Shamsuddin
F. Dazzo
E. Schroeder
Y. Velasquez

INOCULATION RESPONSE

Work group II was first asked to determine whether, and if so, what generalizations about legume inoculation could be drawn from the studies run by work group members. While the group agreed that legume inoculation is important, we felt that it was as important or perhaps more so to focus attention on factors that affect inoculation response. In some cases, trying to obtain an inoculation response in soils where indigenous Rhizobium support an adequate symbiosis may be unproductive. In addition, we strongly urge that all inoculation response trials include adequate controls such as soil tests to determine if other limiting factors are present.

With respect to the questions posed in the questionnaire, we concluded that, for several reasons, it is difficult to generalize either about inoculation response from our own studies, or inoculation studies in general.

First, most of our studies, to date, have focused on strain selection and not on inoculation response. Secondly, response to inoculation depends on a number of interrelated parameters including environment, rhizobial strain, and plant cultivar. In addition, whether or not one concludes an inoculation response has occurred can depend on which measurement parameter is used, i.e., yield or nodule number.

One parameter that the group felt was important to measure, that is rarely included in inoculation resonse studies, is the nitrogen carried over from the previous legume crop and the nitrogen carried over to the following crop.

The group concluded that it was rarely possible to predict an inoculation response because very few studies are large enough in scope to analyze or maximize all the factors involved in the inoculation response. The only time a positive response to inoculation could be reliably predicted was in the rare instance when a legume crop was being grown in rhizobia-free soil.

However, the group felt that if this type of predictive ability was desired, it would be necessary to move beyond mere strain selection studies and conduct experiments. These experiments would include: soils tests, particularly

those for nutritional toxicities and deficiencies; enumeration and evaluation of the indigenous rhizobial population; and use of stringent controls, including at least nitrogen, uninoculated legume, and perhaps non-fixing plant controls.

RECOMMENDED STRAINS & PLANTS

With respect to the use of the rhizobial strains and plant cultivars recommended by the Washington D.C. Workshop of 1982, very few group members had used the recommended strains because very few people knew of the existence of the lists. This seemed to be due to very limited distribution of the workshop proceedings. In fact, most of the recent grantees were completely unaware of the 1982 meeting. Consequently, the group recommended that all new and current grantees be given a list of the recommended strains as soon as possible and that these lists be updated every 3 to 5 years.

With regard to the plant cultivars recommended by the Washington Workshop, the consensus was that for most crops comprehensive cultivar lists were not available and that it may be difficult in any event to recommend one cultivar for all field studies because cultivar performance is so site specific. A better alternative would be to use a cultivar recommended for the region in which the study is being done. The group also recommended that all PIs studying the same crop include one common variety wherever possible as a local check. Most of the grantees had not used the cultivars recommended by the Washington 1982 meeting because they did not know of their existence. Again it was recommended that lists of the recommended cultivars be distributed to grantees and that these lists also be updated every 3 to 5 years.

PROGRAM EMPHASIS

The group felt that while inoculation response studies should be continued as part of the Limiting Factors Program more strain selection studies should not be funded since there are many good strains now available for the major legume crops. The group recommended that studies be done to maximize inoculation response and that such studies should try to define all factors limiting inoculation response in a given country. These studies should focus on evaluating fewer strains over a broader range of environments. In such studies, the strains recommended by AID or NifTAL should be used.

The list of recommended strains should include 3-5 strains for each major legume crop; with each strain adapted to different environmental conditions. The lists should also contain data on the soil type, pH, climate, and cultivar for which the strain is recommended, as well as references on the source of the strain and names of other PIs that have used it.

We concluded that the major constraint to obtaining an inoculation response in the field was competition between inoculant and indigenous rhizobia. Following competition in importance were inoculation methods, water availability, soil factors, plant by strain interactions, and pathogens and disease. In general, these latter constraints cannot be ranked since they are all interrelated in

their effects on inoculation response. The group felt that competition was a serious limiting factor to inoculation response for all crops but in particular for *Phaseolus vulgaris*.

MOLECULAR BIOLOGY

We agreed that advances in molecular biology hold promise for overcoming the problem of rhizobial competition. For example, transposson mutagenesis could be used to evaluate factors controlling competition from both host and rhizobial points of view.

The group was in agreement that the development of promiscuous nodulating plant lines would not solve the problems of variable inoculation response. In fact, they recommended the opposite strategy; that efforts be made to produce plant lines that would nodulate only with the most effective rhizobia. Studies using Rj1 genes might be helpful in this area.

RESEARCH STRATEGY

The experimental program that we felt might lead to the ability to predict inoculation response should include soil analysis, analysis of indigenous rhizobial population, and the use of stringent controls. As many of the limiting factors as possible should be evaluated. Strain selection for maximizing BNF should only be done after we can reliably predict positive inoculation responses.

We were also asked to devise a strategy to assure an LDC farmer a positive response to inoculation. While the group felt that it was impossible to assure a positive inoculation response, we agreed that in order to increase the incidence of positive inoculation responses in a particular developing country we need the following:

- More scientific data including strain and cultivar recommendations
- Knowledge about the effects and availability of realistic economic inputs and outputs, e.g., fertilizers
- Evaluation of different management systems
- Understanding of social constraints, i.e., dietary preferences
- To use simple approaches which are realistic

FUTURE DIRECTIONS

We concluded that if the Limiting Factors Program had to be reduced in size, inoculant technology studies should be eliminated. Our rationale for this choice is that all other limiting factors had to be eliminated before inoculant technology could be justified. Also there are other sources of funds, e.g., industry, to support work in inoculant technology.

We did not feel that the other four topic areas could be ranked as to their importance because they are so integrated in nature. Similarly, we felt that instead of cutting one or several program areas, if cuts became necessary, it would be better to retain all topic areas and fund only the best proposals up to the limit of the funds available. In this way quality research would be retained as a key characteristic of the Limiting Factors Program.

Another recommendation made by the group was that communication should be improved. The program should support the publication of a recommended Rhizobium culture list, as well as a current projects yearly bulletin. This bulletin should include a summary of each new project and main accomplishments of all current projects. This type of publication would facilitate the interchange of information and strengthen collaborative ties between program grantees. Also, a culture collection of the recommended strains should be set up and fundrd by AID.

Lastly, the group felt that the goal of the Limiting Factors Program should be two-fold: short term studies should focus on applied problems. In the course of solving these problems areas for long-term biotech research would be identified. The positive feedback loop generated by integrating the two types of studies could hasten tne elimination of factors that limit the full expresssion of BNF in small farmers' fields in developing countries.

REPORT OF WORK GROUP III ON SOIL FACTORS THAT LIMIT BNF

Work Croup III Members:

P. Graham, Chairperson
D. Hubbell, Rapporteur

S. Abdel-Ghaffar
R. Lockerman
J. Sartain
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LIMITING SOIL FACTORS

Working group members were asked to rank seven soil limiting factors in order of importance. The ranking at which they arrived were--from most to least important:

- Nutrient deficiencies and soil temperature/soil moisture (received equal ranking)
- Ineffective rhizobial strains
- Pesticides and fertilizers
- Acidity, alkalinity, and salinity
- Antagonistic effects of other soil organisms
- Antagonistic effects of plant roots

Consistent with this ranking, nutrient deficiencies were the most common topic addressed by members of the group in their Limiting Factors grants. This topic was probably most heavily emphasized because it is more ubiquitous, more obvious, and more amenable to remedy.

With respect to nutrient deficiencies, we concluded that while phosphorus is almost always limiting in the tropics and is critical for nitrogen fixation, potassium is rarely limiting, nitrogen usually gives a positive yield response, and little is known about the effects of trace element limitations on BNF.

The strategy advanced by the group to overcome the most limiting soil factor (nutrient deficiencies) was fertilization at rates determined by standard greenhouse/field trials.

With respect to the use of the plant cultivar and rhizobial strains recommended by the Washington D.C., workshop, only one of the eight members of the working group used recommended rhizobia in his Limiting Factors research. The other PIs had not used the strains because they were not aware of the existence of the recommended list. Similarly, the cultivars recommended by the workshop were not used by any of the projects and for the same reason.

PROGRAM EMPHASIS

The group felt that the Limiting Factors Program should concentrate more on soil limiting factors because these are the type of limiting factor most commonly encountered. Other soil factors which may be important limiting

factors to BNF are erosion, micronutrients, nematodes, and microbial root pathogens. None of the grants held by the members of this working group have addressed any of these factors.

It was felt that molecular biology might provide ways to overcome soil limiting factors but only in the long term. And, group members questioned that even if molecular biology provided some technological breakthroughs would the research be appropriate or feasible in developing countries.

RESEARCH STRATEGY

The strategy we recommend for overcoming nutrient deficiencies which limit BNF should include developing mechanisms for improving/maximizing utilization of phosphorus and other limiting nutrients by plants. Implicit in this strategy is the caveat that, if farmer's resources are limited, phosphorus should be emphasized in preference to nitrogen and that alternative and less expensive sources of phosphorus should be considered.

FUTURE DIRECTIONS

The group felt that if one of the five topic areas currently funded under the Limiting Factors program had to be eliminated it should be constraints to implementation of inoculant technology. The remaining four areas were ranked in order of decreasing importance:

- Soil factors that limit BNF
- Strain selection and inoculation response
- Plant factors that limit BNF
- Rhizobial factors that limit BNF

However, we do not recommend that any of the five topic areas should be eliminated. If funding constraints require a cutback in the program, it should be accomplished by reducing the number of grants awarded.

We would highly recommend that emphasis in the program should be given to developing technology which is low cost and highly available. Finally, the group felt that the scientific goal of the Limiting Factors program should be to increase legume production by making BNF technology applicable and feasible for subsistence farmers in developing nations.

REPORT OF WORK GROUP IV ON RHIZOBIAL FACTORS, INCLUDING INOCULANTS, THAT LIMIT BNF

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INOCULANT TECHNOLOGY

The major constraint to successful implementation of inoculant technology in developing countries is the demonstration of the need for inoculation to extension agents and farmers. Such demonstrations can help generate farmer support and elucidate the conditions under which inoculation responses can be obtained. To meet this objective, high quality inoculant must be available.

Some of the projects conducted by work group members proved that inoculation of legumes can improve yields. In some cases, the field locations were such that extension agents and farmers were able to see, first-hand, the benefits of inoculation. Similar experiments should be run in more locations so that sufficent information is available to aid in predicting when yield responses can be expected.

A second major constraint to the successful implementation of inoculant technology in developing countries is the availablility of high quality inoculants. Eliminating this constraint would require technically trained personnel who are able to produce and run quality control on inoculants. At the same time, producing high quality inoculants will require carefully selecting carriers, methods of production, inoculation, and culture and inoculant storage.

RESEARCH STRATEGY

In order to demonstrate the response of legumes to inoculation, we propose the following strategy. Field sites should be carefully chosen so that other factors such as fertility or moisture are not limiting. High quality inoculants should be used. These can be prepared by trained personnel. We strongly recommend that at least a few of the personnel be trained at the graduate level. Aside from long term graduate training, short term training courses of at least 6 weeks duration should be available to train technical personnel. These courses should emphasize inoculant production and quality control.

PROGRAM EMPHASIS

The group did not feel that the percent of Limiting Factors projects devoted to inoculant quality and inoculation success should be increased. It was felt that all topic areas were equally important and necessary to provide a balanced approach to maximizing BNF.

If the constraints to the

implementation of inoculant technology were resolved, the group estimated that one could reasonably expect a 2- to 3-fold increase in crop production during the first 3 years. After that, yield increases of 20% would be likely due to the build-up of high populations of inoculant strains in the soil.

RHIZOBIAL LIMITING FACTORS

With respect to other rhizobial factors, the group felt that competition was the major constraint to maximizing BNF. The projects conducted under the Limiting Factors Program, as well as others done by numerous labs worldwide, suggest that strain selection for more competitive strains and use of high inoculation rates to provide high populations of rhizobia can be useful in ensuring that a large number of nodules are formed by the inoculant strain. In addition, one avenue of profitable research may be the development of cultivars that selectively nodulate with highly competitive, high nitrogen-fixing strains.

PROGRAM EMPHASIS

The group did not feel that greater emphasis should be placed on solving the competition problem by the Limiting Factors Program. There is already an adequate balance between topics but additional funding is needed for all topics. We concluded that if the problem of competition could be resolved, it would be reasonable to expect yield increases of 25%. Lastly, we recommend that at least some attention be focused in competition between strains within inoculants.

Plant cultivars and rhizobial strains recommended by the Washington workshop were sometimes but not always used by work group members. Most work group members were unaware of the existence of lists of recommended strains/cultivars.

RESEARCH STRATEGY

We felt that molecular biology held the greatest promise for solving the problem of rhizobial competition, but that the limited resources of the Limiting Factors Program would be more wisely spent supporting research utilizing more conventional approaches. In any event, it was likely that 10 to 20 years of research would be necessary before the molecular biology approach would bear fruit.

Similarly, the group felt that the developent of promiscuous nodulating plant cultivars would only increase and not solve the problems of competition since it is highly unlikely that a wide range of rhizobial strains will all be highly effective on the same cultivar. Also increased promiscuity does not imply that strains are going to be equally competitive on the same legume host.

FUTURE DIRECTIONS

If the Limiting Factors Program has to cut one of the five topics areas, we recommend that soil factors be eliminated because the ways to overcome many of the soil constraints that limit BNF are well known. Of the four remaining topic areas, our ranking of relative importance was:

- Plant factors that limit BNF

- Rhizobial factors that limit BNF
- Strain selection and inoculation trials
- Constraints to implementation of inoculant technology

If it were necessary to cut all but one topic, we recommend that plant factors that limit BNF be retained. Lastly, we believe that the scientific goal of the Limiting Factors Program should be to obtain an effective Rhizobium/legume symbiosis under conditions which normally constrain BNF and hence crop productivity.

Comparison Field

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Isolation and Evaluation of Inoculants for Woody Legumes in Veracruz, Mexico.

I.L. Pepper*, University of Arizona, J.P. Roskoski*, University of Hawaii, and E. Pardo, Instituto Nacional de Investgaciones sobre Recursos Bioticos, Veracruz, Mexico.

Rhizobia were isolated from nodules of Leucaena leucocephala and Acacia pennatula collected from 17 locations in the state of Veracruz, Mexico. These species were chosen because of their multil-use potential. Collected isolates plus additional strains were screened for effectivity in a greenhouse study. The two most efficient isolates, as measured by total plant biomass and nitrogen content were used for the field inoculation study.

Two month-old seedlings of L. leucocephala and A. pennatula, inoculated at the time of nursery establishment with isolates selected in the greenhouse study or field site soil, were outplanted in Jalapa, elevation 1500 m, soil pH of 5.0 and La Balsa, elevation 600m and soil pH of 8.0. Height growth was measured at monthly intervals for 18 months.

Results showed large significant differences in height growth for L. leucocephala between sites. Trees in LaBalsa were three times taller than those in Xalapa, 83 vs 267 cm. Significant inoculation differences were found in Jalapa. Although all trees were nodulated,plants inoculated with TAL 1145, a strain adapted to acid soils, were significantly taller than plants inoculated with either isolate 5B, isolated from an alkaline soil, or the controls. Thus although Leucaena nodulated with the native rhizobia, it benefited from inoculation with an introdulced strain.

In contrast to Leucaena,A. pennatula trees at the two sites had similar heights. As with Leucaena, Acacia showed significant inoculation treatment differences in Xalapa but not in La Balsa. Isolate 16, originally obtained from the same site where the field study was run was superior to both isolate 14 and the control. These differences translated to 2.0 metric tons/ha for plants inoculated with isolate 16 vs 1.5 and 1.0 metric tons/ha for trees inoculated with isolates 14 and the control, respectively. These results indicate that Acacia benefited when an indigenous strain was applied in large numbers as inoculum.

Leucaena nodules, collected in Jalapa at the end of the field study, were occupied by both TAL1145 and 5B, with TAL 1145 dominating. Surprisingly only 5B was found in nodules from the La Balsa site, even in nodules from trees originally inoculated with TAL 1145.

A competition study in sand culture showed that strain TAL1145 and 5B were equally competitive when applied as a mixed inoculum.. However, in a pot study using soils from the two field sites, 5B competed significantly better for nodule occupancy in the high pH LaBalsa soil, while TAL1145 competed better in the acidic Jalapa soil.

Overall, the data show that inoculation of Leucaena leucocephala and Acacia pennatula nursery stock with superior effective rhizobia can result in greater height growth and increased biomass production in some sites.

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Selection for enhanced N_2 fixation in common bean germplasm for Honduras.

F.A. BLISS and J.C. ROSAS, Univ. of Wisconsin, and J.F. CHANG *, Escuela Agric. Panamericana, Honduras.

High N_2 fixation potential from superior bean genotypes, choosen as donor parents, is being incorporated into small red-seeded germplasm suitable for Honduras by using the inbred backcross line method of populations, developed by using the local cultivar Desarrural as the recurrent parent, have indicated that several inbred backcross lines had better nodulation and higher yield than local check cultivars, and also suggested that some improvement for both traits was possible in these populations. Moreover, desirable plant and seed traits from the local cultivar Desarrural have been recovered in these populations. Currently, superior disease resistance, yielding ability and plant type, are being incorporated into lines selected from these two populations by utilizing superior red-seeded genotypes instead of local cultivars with poor agronomic performance. Also, these red-seeded lines are being crossed with high N_2-fixing donor parents to develop new breeding populations for Honduras. Superior red-seeded genotypes are being identified by continuous testing of genetically-diverse germplasm obtained from different sources. Simultaneously, inoculation trials have been initiated at several sites, in collaboration with researchers from Honduras, to study the response to inoculation in field conditions similar to those where commercial beans are produced and where advanced breeding lines will be tested in the near future.

Chickpea Response to Various Soil Moisture Levels.

R. H. Lockerman*, J. R. Sims, G. W. Westesen[1], F. J. Muehlbauer, J. Hall[2], A. S. Abdel-Ghaffar, and H. El Attar[3], Montana State University[1], Washington State University[2], and Alexandria University[3].

A line-source sprinkler irrigation system was utilized to superimpose four moisture levels over three dryland sited in Egypt, Washington and Montana. Genotypes planted at right-angles to the applied water source were evaluated for plant growth and seed yield. ILC 591 had the highest consistent seed yield at the high soil moisture level at all three locations in 1984. However, germination of ILC 591 was poor in Egypt. ILC591, UC-5, ILC523, ILC 294, and ILC 171 had the highest yield at the high soil moisture level in biological yield at the high soil moisture level in Pullman, Washington. Overall yields of all genotypes at Bozeman, Montana were suppressed by Ascochyta. Desi type genotypes were more resistant to Ascochyta.

Rhizobia and Mycorrhizae to Enhance BNF in Cowpeas.

L. S. WAMOCHO*, H. D. SKIPPER, and W. L. OGLE; Egerton College, Njoro, Kenya and Clemson Univ., Clemson, SC, USA.

The goal of this research is to help alleviate poverty conditions of the Eastern and Western Provinces of Kenya through improved diet by increasing BNF and cowpea yields. Specific objectives are:

1. Determine nodulation and yield response of cowpea cultivars to rhizobial strains in non-fumigated soil.
   a. Greenhouse: 3 cultivars by 5 rhizobial strains
   b. Field: 2 cultivars by 2 rhizobial strains

2. Determine nodulation and yield response of cowpea cultivars to mycorrhizal fungi.
   a. Greenhouse: 3 cultivars by 4 mycorrhizal fungi in fumigated and non-fumigated soil.
   b. Field: 2 cultivars by 2 mycorrhizal fungi in non-fumigated soil.

3. Evaluate yield response of 2 cowpea cultivars to 2 rhizobial strains and 2 mycorrhizal fungi under field conditions.

4. Isolate, culture, and evaluate 4 to 6 native mycorrhizal fungi from Kenya.

5. Compare 2 to 4 of the VAM fungi from Kenya with 2 to 4 of the "stock" mycorrhizal fungi from Clemson for infection potential and cowpea fields.

Acid soil tolerance of bean cultivars and rhizobia.

P. H. GRAHAM, A. A. VARGAS. (University of Minnesota, St. Paul) and C. ESTEVEZ SALAZAR (INIAP, Ecuador)

Beans (*Phaseolus vulgaris* L.) are a staple crop throughout much of Latin America, and often grown in marginal soils of low pH. This can limit nodulation and N_2 fixation. This study aims 1) to evaluate cultivars of *P. vulgaris* for tolerance of pH. 2) to collect and evaluate strains for tolerance of acid pH. 3) to study strain persistance and competition in acid soils. 4) to determine the basis for host and strain differences in pH tolerance, and 5) to evaluate inoculation method and its importance in acid soil.

278 strains of *R. phaseoli* have been isolated from bean-producing regions in Ecuador. Surprizingly, most soils sampled were not particularly acid. 270 strains including 70 from those obtained above, plus additional isolates from Brazil and Mexico were evaluated for pH tolerance using an agar plate method. 75 have been shown tolerant of pH 4.5 and 31 tolerant of pH 4.5 + 100μ M Al. 24 of these isolates are also effective in N_2 (C_2H_2) fixation. Eleven of 126 culivars evaluated were tolerant of pH when dependent on symbiotic N_2 fixation for growth. These included 'Prero 143' (3 sources) 'Capixaba precoce', Bat 76 and RIZ 54. Crosses have been made of sensitive x tolerant cultivars to determine the inheritance of this reaction.

When cultivars, tolerant to or intolerant of low pH were paired with pH sensitive or tolerant strains, marked differences in nodulation response were obtained. With the tolerant cultivar "Capixaba precoce" nodulation was adequate with both the pH tolerant strain UMR 1899 and with the sensitive UMR 1632. With 'Negro Argel', a cultivar sensitive to pH, nodulation was obtained only with UMR 1899. These differences did not appear related to multiplication effects in the rhizosphere or to problems in attachment.

Factors affecting the competitive ability of isolates of Rhizobium phaseoli.

P. H. Graham, L. A. OLIVERIRA (Univ. of Minnesota, St. Paul) and C. VIDOR, UFRGS, Porto Alegre, Brazil.

Beans (Phaseolus vulgaris L.) are a staple crop throughout much of Latin America, and most soils contain high numbers of infective, though not necessarily effective, R. phaseoli. This can constrain N_2 fixation. This project aims 1) to determine the population of R. phaseoli in Brazilian bean soils 2) to identify strains of R. phaseoli differing in competitive ability, and to examine reasons for this difference 3) to evaluate bean cultivars for ability to select particular strains from a mixed environment, and 4) to examine their importance of environmental factors in competition.

Most probable number (MPN) counts of R. phaseoli in 52 soils from Rio Grande do Sul varied from log 1.00 to log 5.73 g^{-1} dry soil, with 50% of the soils showing counts in excess of 10,000 cells g^{-1} dry wt. soil.

A method has been developed for the rapid screening of strains for both competitive ability and N_2 fixation. When UMR 1116, a nod^+ nif^- variant of CC 511 was mixed with UMR 1899, an effective strain of R. phaseoli in ratios varying from 1:16 to 16:1, plant dry weight production was positively correlated with the level of UMR 1889 in the inoculant mixture. ($y = 0.027x + 2.9$, $r = 0.87$). Using UMR 1116 in a 1:1 ratio with test strains, and applying 10^5 cells/g of soil, 80 strains of R. phaseoli (selected from 200+ isolates obtained from 8 different states in Brazil on the basis of pH tolerance) have been screened for competitive ability with UMR 1116. 30 strains showing effective and competitive response under these conditions have been selected for field trials. Several of these were included in inoculation trials in the Brazilian cerrado in 1985.

Soil Factors Limiting Nitrogen Fixation in Beans in Honduras

D. H. Hubbell, J. B. Sartain*, A. M. Andrews, Univ. of Florida.

Field and glasshouse research was undertaken to identify major soil factors limiting N fixation in dry beans under tropical conditions in Honduras and to determine appropriate management practices to correct these limiting factors as a basis for practical recommendations for bean cultivation by small farms. To date the influence of soil acidity and moisture and applied P and Mo on the growth and yield of different dry bean cultivars have been investigated. Cultivars have responded differently to the application of lime and selected Rhizobium strains. Positive yield responses were noted for increasing rates of lime on particular soils, but no response to P has been variable and to Mo non-significant. Most cultivars did respond to applied N.

Potential of Increasing Cowpea Production in the Caribbean by Inoculation.

R. W. WEAVER* AND RICHARD GRAHAM, Texas A&M Univ. and Caribbean Agric. Res. and Development Institute Barbados Unit.

The overall objective of this research is to obtain the data required in determining the potential of cowpeas grown in Barbados to respond to inoculation. Two approaches will be utilized to obtain the needed data. One approach involves characterizing the rhizobial population in the Barbados soils on the basis of size and effectiveness in nitrogen fixation. The other approach involves growing inoculated cowpeas in the field and measuring plant growth response. Fundamental to the first approach is the assumption that if populations of rhizobia capable of nodulating cowpea are low in soils or if they are of low effectiveness in N fixation, there is potential for increasing cowpea yield by inoculation. This approach will provide basic data for many fields in Barbados and can be used in determining the quantity of inoculum that must be added to the soil to provide potential for inoculant rhizobia to successfully compete with soil rhizobia to form nodules. It will also result in isolation of effective strains of rhizobia that may be particularly well adjusted to soils in Barbados and popular cowpea varieties. The second approach to be utilized involves field experimentation to determine the actual response to inoculation since field response of cowpeas to inoculation is the ultimate goal of the proposed research. The limitation to this approach is very few locations can be evaluated and as is typical for field experimentation the research results are greatly influenced by weather conditions and soil variability. Since only four locations can be tested each of two years it is essential that the field sites for testing be carefully selected to represent the different rhizobial populations. Also, the strains used for inoculation should be the best available. The results obtained will only have meaning for the strains used in inoculations. When better strains are obtained, the field research may need to be conducted again. The basic rhizobial population data obtained from the survey will always be of benchmark use. By using the two approaches together, a very good basis can be formulated during the relatively short duration of the project for recommending inoculation of cowpeas to farmers.

Competition between Rhizobial Inoculants and Indigenous Rhizobia

D. D. Focht, University of California, Riverside, B. S. Hernandez, Universidad de Panama

A BNF study was conducted in 55 gallon drum lysimeters which contained ^{15}N enriched soil organic N by virtue of a previous 18-month incubation with 20% excess atom % ^{15}N. Plants were inoculated with 4 different strains, 2 of which were Hup^+ and 2 of which were Hup (See Table 1). No discernible differences in ^{15}N enrichment were observed between the treatment. The atom % ^{15}N of the harvested plants (0.399 ± 0.008). BNF cannot be determined at this time until the ^{15}N data have been analyzed for the non-fixing control plant (eucalyptus). P 132 nevertheless was an excellent competitor in terms of nodule occupancy and an effective yield producer (Table 1).

TABLE 1. Effect of inoculum strains on seed yield.

	g plant^{-1}		ANTIBIOTIC TYPING 1/	
STRAIN	x	s	x/n 2/	p 3/
P132	94.5	±23.5 a	61/61	1.0 a
IHP147	63.5	±28.1 b	39/50	0.78 b
22A1	57.9	±19.8 bc	29/60	0.48 c
401	57.2	±21.1 bc	10/44	0.23 d
Control	44.8	±12.8 c	o/75	0 e

1/ multiple (total of three) resistance

2/ x = number of resistant nodules
n = total number of nodules

3/ frequency

Effects of Phosphorus fertilization on Symbiotic nitrogen fixation

Vicia faba, A. S. Abdel-Ghaffar, J. R. Sims, H. A. El-Attar, R. H. Lockerman, and M. H. El-Halfawi, Alexandria University (Egypt) and Montana State University (USA).

Field experiments were conducted on the effects of P fertilization on nodulation and symbiotic N fixation potential of Vicia faba (Giza 1) in clay and sandy clay loam soils nearby Alexandria, Egypt. Five levels of P (0, 35, 70, 105, 140 and 17 kg P_2O_5/ha) with and without 70 kg N/ha. were used. The obtained results could be represented by the following two experiments. Experiment 1: Clay soil, season: 1980-1981 The number, and dry wt. of nodules, $N_2(C_2H_2)$ fixation, dry matter, N and P contents of plant shoots and seed yield decreased by increasing the rate of P fertilizer from 70 to 140 kg P_2O_5/ha. Addition of 70 kg N/ha as ammonium nitrate reduced the nodule number and dry wt. and N_2 (C_2H_2) fixation but resulted in

increasing the dry matter, N and P contents of plant shoots and seed yield. Application of P fertilizer just before cultivation was much better that at 74 days after sowing with respect to all variables tested. Also, triplephosphate proved to be superior to superphoshate. Experiment 2: Sandy clay loam soil, season: 1981-1982 The number and dry wt. of nodules, $N_2(C_2H_2)$ fixation, dry matter, N and P contents

of plant shoots and seed yield increased with P fertilization up to 105 kg P_2O_5/has and decreased with higher levels. Under the experimental conditions, high P levels not only partially inhibited nodulation and nitrogen fixation but also plant growth.

Improvement of Nitrogen Fixation and Yield of Lentils and Chickpeas in Turkey

L. Cakmakci and D. F. Bezdicek University of Ankara, Turkey and Washington State University.

During trips to Turkey in 1982 and 1984, approximately 500 isolates of Rhizobium from lentil and chickpea were taken throughout Turkey. Nodulation of lentil was generally poor in Turkey, with a substantial portion of the nodules being infested with larvae of the satona larvae. Accompanied soil samples were analyzed for soil organic matter, pH, salts, and $CaCO_3$. From a pre-screening of isolates, a minimum number were tested both in Turkey and Washington and later evaluated in the field in addition to isolates from other sources. Greenhouse evaluations showed that the Turkish lentil isolates to be generally superior to isolates from Washington, commercial sources and from wild species when inoculated on both Washington and Turkish cultivars. Field studies conducted on selected isolates showed significant increases in dry matter production, but not in yield for selected larvae. No statistically significant relationship was found between the incidence of infestation and seed yield. Studies conducted at other locations (U.S. and Turkey) on selected isolates did not show a significant increase in yield. Isolates taken from wild lentil cultivars in Turkey and Europe were inferior to isolates taken from cultivated species in Turkey. Several sources of peat carriers were identified in Turkey and were compared for survival of rhizobia in carriers from the U.S. Results showed that the sources in Turkey are comparable to those in the U.S. with respect to survival of Rhizobium.

Meloidogyne spp. as Limiting Factors on N_2 Fixation by Forage Legumes in the Tropics. D. D. BALTENSPERGER*, K. H. QUESENBERRY, S. G. TAYLOR, and V. W. SAKA, Univ. of Florida and Dept. of Agric. of Malawi.

Root-knot nematodes (Meloidogyne spp.) are frequently a significant pest of forage legumes in tropical and subtropical areas. Screening techniques have been developed to evaluate the response of large numbers of tropical forage legumes to root-knot nematodes. These studies evaluated the interaction of root-knot nematodes with plant genotypes (varying levels of resistance) with nodulation, plant N content, and yield. Separate experiments were conducted on alyceclover (Alysicarpus vaginalis L. DC.) and Aeschynomene (Aeschynomene americana L.) combined with desmodium (Desmodium heterocarpon L. DC.) to evaluate these interactions. Under no to moderate root-knot nematode (Meloidogyne arenaria, M. javanica and M. incognita) stress imposed on alyceclover, no significant interactions were detected between nematode treatments and cultivars for yield, N content or nodulation. There was a significant (0.05) interaction for gall score. Cultivars were significantly different for all variables monitored. Aeschynomene and desmodium were subjected to similar treatments, but the plots were more severely stressed by cutting the plants back once. The interaction of nematode treatments and cultivars was significant for yield, N content and gall score but not nodulation or end of the season acetylene reduction. Severe root-knot infestation often resulted in death of susceptible plants prior to the end of the season. From these studies and other observations we conclude that there is very little if any direct effect of these three root-knot nematode species on nodulation or nodule activity. Indications are that reduced N-fixation in the presence of these root-knot nematode species is associated with reduced nutrient uptake and subsequent reduced photosynthesis, or root deterioration associated with gall break-down and secondary infection.

Peanut inoculation responses in North Cameroon.
T.J. Schneeweis *, J.C. Wynne, G.H. Elkan, and T.T. Schilling, North Carolina State University and Institut de la Recherche Agronomique, Cameroun.

Peanut (Arachis hypogaea L.) and Bradyrhizobium spp. inoculation trials were conducted in semi-arid North Cameroon during 1983 and 1984. Inoculum was prepared by injecting YEM-grown cells into packets of gamma irradiated peat. For each strain, the weight of inoculant added to water was adjusted to give a cell density of 1.6 X 10^6/ml. The peat-water mix was added to the open row at a rate of 10 ml per 15 cm. of row. In 1983, three cultivars and three strains were used. The combination of cv. 28-206/NC92 gave a 26% yield increase over the uninoculated control at one of the two locations. In 1984, each of four host X strain tests consisted of four promising cultivars for each of two regions and five greenhouse-selected strains shown to be compatible with these cultivars. Significant differences existed between locations, among cultivars, and among strains for pod and seed yields. Seed and haulm yield for the nitrogen control (196 kg urea/ha) were not significantly differeent than some host X strain combinations, showing that optimization of BNF can effectively substitute for nitrogen fertilization in these environments.

Stability of Effectiveness in Cowpea Rhizobia. R. W. WEAVER, NANTAKORN BOONKERD, AND HARRI PERSAUD, Texas A&M Univ., Thailand Dept. of Agric., and Guyana Ministry of Agric.

Inoculation of a soil with rhizobia puts them under various environmental stresses that leads to selection of subpopulations that may not be as effective as the original population. The hypothesis was tested by incubating different strains of rhizobia in a sandy pH 5.7 soil obtained from Thailand. Three strains of cowpea rhizobia were utilized; two nodulated peanuts, Tha-201 and T-1; and one nodulated cowpea, TAL-309. The rhizobia were incubated under dry and moist (-0.33 bar) soil conditions at 40°C in sterilized soil and at 45°C in nonsterilized soil. Eighty single colony isolates of each strain were obtained from nodule isolations of cowpea and peanut which were inoculated with soil samples immediately following soil inoculation and 15 days later for nonsterilized soil. Single colony isolates were also obtained from pour plates of soil dilutions of sterilized soil immediately following inoculation and again after 45 days of incubation. Results indicated that there were changes in effectiveness of some strains due to treatments. Relatively ineffective isolates (<60% of plant dry matter produced as compared to parent culture) were found in relatively high frequency (30% of isolates) in the population of nodule isolates of strain TAL-309. The variation was not as great when strain TAL-309 isolates were obtained from soil. Strains Tha-201 and T-1 were comparatively stable in effectiveness. Selection of strains that have stable effectiveness characteristics is important for inoculant production.

Successful inoculation of peanuts and cowpeas depends on the survival of rhizobia in soils which fluctuate between wide temperature and moisture extremes. Survival of two cowpea rhizobial strains (TAL 309 and 3281) and two peanut rhizobial strains (T-1 and 201) was measured in two soils under three moisture conditions (air-dry, moist (-0.33 bar), and staurated soil) and at two temperatures (25 and 35°C) when soil was not sterilized and at 40°C when soil was sterilized. Populations of rhizobia were measured periodically for 45 days. The results in nonsterilized soil indicated that strain 201 survived relatively well under all environmental conditions. The 35°C temperature in conjunction with the air-dry or saturated soil was the most detrimental to survival. At this temperature, the numbers of strains T-1, TAL309, and 3281 decreased about 2 logs in dry soil and 2.5 logs in saturated soil during 45 days of incubation. In sterilized soil, the populations of all strains in moist soil increased during the first 2 weeks, but decreased rapidly when incubated under dry conditions. The populations did not decline under saturated soil conditions. From these results it appears that rhizobial strains to be used for inoculant production should be screened under simulated field conditions for enhanced survival before their selection for commercial inoculant production.

Competitive Nodulation and Growth of Cowpeas. R. W. WEAVER*, PRECHA WADISIRISUK, and AUDREY TROTMAN, Texas A&M Univ., Thailand Dept. of Agric., and Guyana Ministry of Agric.

Knowing the number and effectiveness of cowpea rhizobia in soil provides a basis for predicting the potential response of legumes to inoculation. Soil in fifteen fields in Guyana were sampled to determine the population size of cowpea rhizobia present and their effectiveness. The population size was influenced by the presence of cowpea in the cropping sequence and ranged from <1 to 10 million rhizobia per gram of soil. Most of the fields contained approximately 200 rhizobia per g. A total of 65 rhizobial isolates were tested for effectiveness and most were only moderately effective and approximately 20% were ineffective. The results indicated that the indigenous cowpea rhizobial populations were low enough in number and effectiveness for a potential response to inoculation of cowpeas grown in Guyana.

Biological nitrogen fixation is considered an important trait of cowpeas (*Vigna unguiculata* (L.) Walp. var. 'California Blackeye' No. 5) for economical production yet the process does not alone provide the quantity of nitrogen required by the plant for maximum productivity. Two experiments were undertaken to determine the potential of an increase in nodule mass and number of bacteroids resulting in increased nitrogen fixation. Cowpeas were grown in a glasshouse for 7 weeks under conditions forcing near total dependence on biological nitrogen fixation for growth. Nodule mass on the roots was varied by inoculating seeds with various ratios of effective and ineffective rhizobia that could be identified serologically and by the color of the nodules. The results of both experiments demonstrated a linear relationship between total nodule mass formed by the effective rhizobia and quantity of nitrogen fixed. The coefficient of determinations were high in both experiments (r^2 = 0.98 and 0.83). The relationship between total nitrogen fixed and total number of bacteroids of the effective strain was not consistent. In one experiment the coefficient of determination was 0.86 but in the other experiment it was 0.42. From these results it appears that there is good potential for increasing nitrogen fixation in cowpeas by increasing nodule mass. An increase in nodule mass would also result in an increase in the number of bacteroids.

Genetic Variability for N_2 Fixation in Vigna Species.

G.C.J. FERNANDEZ* and J.C. MILLER, JR., Texas A&M Univ.

Cowpea (Vigna unguiculata (L.) Walp.) and mungbean (Vigna radiata (L.) Wilczek) have the ability to fix N_2 in association with the cowpea miscellany group of Rhizobium spp. This symbiotic relationship involves a complex interaction among the host plant, microsymbiont and environment. We have observed differential N_2 fixation expressed as nodulation and nitrogenase activity in both cowpea and mungbean. Diurnal and seasonal patterns of N_2 fixation were also studied, and nitrogenase activity peaked at 1200 h in both species. In cowpea, narrow sense heritabilities (h^2) for nodule number and nitrogenase activity were 0.55 and 0.62, respectively, for a F_2 population obtained by crossing high and low fixing cultivars. Heritability estimates were influenced not only by the host genotype but also by the rhizobial strain used. In similar studies with mungbean, h^2 estimates for nitrogenase activity were high with strain 31Z3 (0.70) and low with strain 41Z2 (0.13). Nodule size was the major component influencing nitrogenase activity in cowpea, while nodule number influenced nodule weight and nitrogenase activity in mungbean. In cowpea, rhizobial inoculation significantly increased seed yield which was the same or greater than that obtained from 100 kg/ha N fertilizer. A measure of genetic divergence among cowpea genotypes using canonical discriminant analysis and Mahalanobis distances confirmed that significant amounts of genetic diversity exist for N_2 fixation components in cowpea. A modified backcross-inbred breeding method is presented as a means for introducing superior N_2 fixing genes into adapted cultivars.

Increasing Phaseolus vulgaris yields in the Dominican Republic by optimizing the host/strain (Rhizobium phaseoli) symbiosis.

E. Schroeder* and R. Martinez Richez, University of Puerto Rico and Secretary of Agriculture, Dominican Republic.

The cultivation of dry beans (Phaseolus vulgaris) represents the majority of grain legume production in the Dominican Republic, which includes cowpeas (Vigna unguiculata) and pigeon peas (Cajanus cajan). Yields are typically low, averaging between 700 and 900 kg/ha. Total production is insufficient to meet local demand, and yield increase/ha becomes an imperative. Given the limited resources of agriculture on small farms in the region, bean inoculation is an alternative to the use of expensive nitrogen fertilizers. The objectives of the proposed research include: 1. Field trials of P. vulgaris cultivars to determine yield response to inoculation with selected strains of R. phaseoli using minimum and optimum fertility levels; 2. Evaluation of the competitive ability of introduced R. phaseoli inoculant strains.

Nitrogen Fixation of Peanuts in Malaysia. A. W. FAIZAH and J. C. WYNNE,* Rubber Research Institute of Malaysia and North Carolina State Univ.
The goal of this research was to increase nitrogen fixation and productivity of the peanut (*Arachis hypogaea* L.) grown as an intercrop among rubber trees in Malaysia. Four single strain inoculants (NC6, NC70.1, 3G4b20 and RP182-13), prepared based on compatibility with Malaysian peanut cultivars, were tested with local inoculants in two field environments at the Rubber Research Institute in Malaysia (RRIM) to determine the need for inoculation of peanuts grown under rubber tree culture. Inoculation produced greater nodulation and increased seed yield although the yield increase was only significant in one test. Three additional field tests at RRIM indicated that the genotype, inoculant and fertilizer application influenced nitrogen fixed by the peanut. Four additional field experiments were conducted in the peanut-growing area of Malaysia to evaluate the effect of rhizobial inoculation (strains NC92, NC6, Bidur 1) on cultivar V-13. Inoculation increased nodulation with NC92 giving the highest nodulation at all sites. Yield was increased by inoculation although the increase varied with location. Applied nitrogen did not significantly improve growth or yield. Greenhouse studies showed that optimum pH for growth and nodulation for peanut ranged from 5.9-6.3. Peanut was more acid tolerant than mung bean, cowpea, and pigeon pea. Significant variation among peanut genotypes occurred for relative growth at pH 3.9 and 5.3. These studies suggest that both nitrogen fixation and productivity of the peanut can be increased by inoculation although the results are not always consistent.

Small Scale Farmer Storage and Hand Application of Granular Soil Inoculant. R. S. SMITH* and C. RAMIREZ M., The Nitragin Co., Inc. and Univ. of Cost Rica.
Research was undertaken to develop an improved inoculant storage method for small scale farmers in the tropics. Several shallow below ground inoculant storage systems were evaluated. A plastic container has been chosen because it is waterproof, chemically inert and stable, insulates, protects inoculants from insects and animals and is readily available and inexpensive. This inoculant container fitted with a wooden lid is placed in a plastic bag with a layer of dry sawdust around the container as further insulation. A twenty (20) cm wet gravel jacket is established around the unit to facilitate cooling. This unit placed under shade and 25 cm below ground provided the best protection inside the container with a stabilized temperature of 25.5°C when the ambient air temperature reached 36.7°C. Inoculant longevity trials within this unit are being conducted. The second objective is to develop a hand applicator for the delivery of granular soil inoculant in both furrow and hill planting systems. A prototype applicator with a capacity of 1.5 kg of granular inoculant has been built and proven successful in preliminary trials. The unit weighs approximately 1.3 kg, with overall length less than 65 cm and features remote on/off hand controls.

Increasing Nitrogen Fixation of Intercropped Peanut in Malaysia and the Philippines. J. C. WYNNE,* North Carolina State Univ.; R. M. ABILAY, Univ. of Philippines at Los Banos and Z. SHAMSUDDIN, Universiti Pertanian Malaysia. Adapting the peanut (Arachis hypogaea L.) to partially shaded conditions could allow it to be intercropped providing income to the grower and supplying nitrogen for growth of the primary crop. Research designed to isolate rhizobial strains that optimize symbiotic nitrogen fixation and yield under conditions of high soil temperature, acidity and high aluminum concentration and to identify specific peanut genotype-rhizobial strain combinations for partially shaded environments has been initiated. Several rhizobial strains have been shown to nodulate and fix nitrogen in symbiosis with peanut cultivars adapted to Southeast Asia in greenhouse studies at North Carolina. These strains are being evaluated for their tolerance to high soil temperature and acidity. A factorial experiment of peanut cultivars UPL Pn4, UPL Pn2 and CES 101, three nitrogen fixation treatments (inoculated, uninoculated and nitrogen control) and two shading treatments (none, 40% shade) is being conducted to evaluate the effect of shade on nitrogen fixation. In addition, 40 peanut genotypes are being screened for shade tolerance. Results should increase the potential for the peanut to be intercropped with coconut or rubber trees providing income to the grower and nitrogen to the primary crop.

Use of Legumes for N_2-Fixation, Forage Quality, and Erosion Control in Nepal. J. H. BOUTON* and D. R. PRADHAN, Univ. of Georgia and Central Livestock Development Centre, Ministry of Agriculture, His Majesty Government of Nepal.

This is a new research project and experiments conducted in the future will include the following objectives: (1) cultivars of perennial forage legumes will be tested for N_2-fixation, persistence, and forage yield at 2 areas in Nepal, and (2) the effect of Rhizobium inoculation to enhance these traits will also be investigated. To study these objectives, replicated experiments will be established at two areas in Nepal; Mustang (10,000 ft) and Jiri (7,000 ft). Species to be tested are Trifolium repens and Medicago sativa. A limited number of cultivars within each species will be used. Stand counts will be made after establishment and on a yearly basis. Aboveground forage will be harvested by hand at appropriate harvest intervals and weighed and a subsample dried for % dry wt. Inoculation experiments will be conducted with NifTAL and will follow the INLIT trials designated 'Experiment A'. The best cultivar from initial observations in objective 1 will be subjected to three Rhizobium treatments: (a) plants inoculated with Rhizobium, (b) plants not inoculated, and (c) plants not inoculated, but supplied with nitrogenous fertilizer. These three treatments will also be placed in factorilized combination with two basic fertility treatments: (a) farm fertility (b) maximum yield fertility. N_2-fixation and inoculation response will be determined by comparing forage yield in the inoculated plots with that obtained in the nitrogen fertilized plots and the unfertilized, noninoculated plots.

Production and conservation of fixed nitrogen in tropical cropping systems: Interaction between management and environment.

P. Singleton, C. Van Kessel*, and C. Escano, University of Hawaii and Philippine Council for Agriculture and Resources Research and Development.

The three main objectives of the study are integrated so that the interactive effects of factors affecting BNF in tropical cropping systems can be examined. Specifically these objectives are:

1. To determine how native rhizobial populations affect our ability to enhance BNF by inoculation. Native soil populations will be characterized for their ability to meet plant N requirements under varied yield potential.

2. Determine how two management variables (rhizobial inoculation and phosphorus fertilization) affect yield potentials and the amount of nitrogen fixed by four legyumes in two environmentns. Experimental sites will resemble each other in terms of soil type and P requirements but differ in elevation and soil temperature. The soil temperature will affect soil N mineralization rates and differentially affect yield and demand for N by the legume species.

3. Determine the potential N contribution of legumes to subsequent crops as affected by management and environmental components of the previous two objectives.

Improvement of Tropical Pastures in Venezuela with Nitrogen Fixing Legumes

A. J. SEXSTONE* and W. B. BRYAN, West Virginia University.

Agreements have been signed with the Universidad Nacional Experimental de Los Llanos Occidentales Ezequil Zamora (UNELLEZ) and Fundacion Servicio para le Agricultor (FUSAGRI) to carry out laboratory, greenhouse, and field research on nitrogen fixation in tropical pastures during 1985-1987. The objectives of experiments are: 1) to develop appropriate *Rhizobium* inoculum to increase N fixation by native and introduced forage legumes in Venezuelan pastures, and 2) to evaluate the establishment, production, persistence, and biological N fixing capability for promising grass/legume tropical pasture mixtures. Initially, experiments will involve the legumes: *Indigofera hirsuta*, *Alysicarpus vaginalis*, *Centrosema pubescens*, *Pueraria phaseoloides*, and *Macroptilium atropurpureum*. Strains of *Rhizobium* will be isolated from legumes growing in the field in Venezuela. These native strains will be evaluated for competitive ability to nodulate in native soils and their effectiveness will be compared with *Rhizobium* strains recommended by NifTAL, CIAT, IVIC, and EMBRAPA. This work will be carried out by UNELLEZ and WVU. In field experiments the same legumes will be evaluated for effectiveness of inoculation using the following treatments: inoculated legume, noninoculated legume plus N, and noninoculated legume. Additional field experiments will examine the legumes in associations with the grasses: *Andropogon gayanus*, *Brachiaria decumbens*, and *Digitaria swazilandensis*. Herbage yield, contribution of grass and legume to herbage yield, persistence of grass and legume and nodulation of legumes will be evaluated. Plans have been made to start experiments in 1985 at three Venezuelan sites.

Biological Nitrogen Fixation in Co-Evolved Rhizobium and Medicago falcata Ecotypes

D. A. JOHNSON*, M. D RUMBUAGH, and NOOR MOHAMMAD, USDA-ARS, Utah State Univ. and Pakistan Agric. Res. Council

Seeds and nodules from diverse ecotypes of M. falcata will be collected from its native habitats in northern Pakistan. These diverse collections will be evaluated initially for their general agronomic performance in field trials in Pakistan. In addition laboratory studies will evaluate effectiveness for nitrogen fixation of the M. falcata ecotypes and associated Rhizobium isolates in comparison with commercially available M. falcata and M. sativa cultivars and commercial strains of Rhizobium meliloti. This will allow the identification of the most consistently effective macro and mircosymbiont combinations and evaluate if coevolution has produced legume-Rhizobium combinations that are more effective in fixing nitrogen than commercially available cultivars and Rhizobium strains. Steptomycin-resistant mutants of the five most effective Rhizobium isolates will be produced and tested with the most agronomically promising M. falcata ecotypes in field trials in northern Pakistan. Nodules from these field-grown plants will be collected and Rhizobium from these nodules will be cultured on steptomycin culture medium to determine the proportion of nodules infected with the selected Rhizobium strains. This will allow an evaluation of their competitiveness with indigenous Rhizobium as compared to commercially available cultivars of M. falcata and commercial Rhizobium meliloti strains. This work will be a major first step in developing legume-Rhizobium combinations for improving rangeland forage production in northern Pakistan.

Improving the Bean-Rhizobium Symbiosis in Mexico.

F. Dazzo*, M. Valdes, and J. Maya Flores, Michigan State University, National Politechnic Institute, Mexico, and Celaya Technological Institute, Mexico.

The major limiting factors in the bean growing region of Celaya, Mexico are ineffective indigenous bean rhizobia and limiting soil nitrogen. The long-range goal of this project is to increase bean production with the use of competitive, effective rhizobial inoculants under Celaya field conditions. The objective is to identify superior nitrogen-fixing combinations of beans Phaseolus vulgaris L. and Rhizobium phaseoli at the major bean growing sites in Celaya. The approach would be to evaluate inoculant responses of two preferred local bean varieties (Flor de Mayo, Canario) and a new bean line (21-58) developed by F. Bliss (University of Wisconsin) which is improved in root nodulation and nitrogen fixation. We plan to compare 9 recommended competitive, effective strains of R. phaseoli under two growing seasons (July without irrigation and Februrary with irrigation). Inoculants would be prepared in irradiated peat and coated on seeds just before planting. Controls would include uninoculated unfertilized plants and uninoculated plants plus N fertilizer (urea & ammonium nitrate combination at 60 kg/ha). Inoculation responses would be compared by color photography, plant dry weight, and nitrogen content, seed yield, and seed nitrogen. Sampling of each bean variety would be at the same stage of development regardless of plant age. The interstrain competition of the highest 2 to 3 yielding strains would be assessed under field conditions during years 2 and 3 of this project.

Factors Limiting Bean Nodulation and Yield in Northern Mexico.

W. C. LINDEMANN, New Mexico St. Univ.

Factors limiting bean (*Phaseolus vulgaris*) nodulation, nitrogen fixation, and seed yield were investigated. Field plots were established under dryland conditions in the northern plateau region of Mexico. *Rhizobium phaseoli* inoculation, inoculation persistence, *R. phaseoli* strains, phosphorus fertilization, and cultivar nodulating ability were investigated as limiting factors. Field plots were also established under irrigated conditions to investigate initial irrigation timing on bean nodulation and inoculant strain survival in southern New Mexico. Bean inoculation dramatically increased seed yields the year of inoculation and the year after inoculation. By the third year after inoculation, yield differences were small between inoculated and uninoculated plots. Inoculation replaced at least 40 kg N/ha. Differences between strains were observed. However, these differences were dependent on year of inoculation and years after inoculation. Phosphorus fertilization also dramatically increased seed yields. Both phosphorus fertilization at 40 kg/ha and inoculation should be recommended practices. Some of the 50 bean isolines had greater nodulating ability than others. Additional screening of bean isolines or varieties is needed to select a line with the genetic potential to produce more nodules. Initial irrigation treatment had little effect on bean nodulation or inoculant strain recovery. Factors limiting bean nodulation and yield in the northcentral plateau region of Mexico are 1) the lack of indigenous *R. phaseoli* and 2) phosphorus fertility.

Fluorescent Rhizobium

APPENDIX

CRITIQUE

"BNF Limiting Factors Grant Program - Past,

Present and Future"

by

D. H. Hubbell

Department of Soil Science

University of Florida

Gainesville, Florida 32611

presented at

BNF Limiting Factors Workshop - 10th NARC

NifTAL Project

Maui, Hawaii

August 11-18, 1985

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Historical Background

A research grant program to fund studies on "Factors Limiting Symbiotic Nitrogen Fixation for Crop Production in Developing Countries" was initiated in 1976, with funds from AID being allocated to CSRS-USDA for administration/distribution as sub-grants. Program funding for 1984 was $500,000. Areas to be emphasized were:

1. Factors limiting inoculant quality and inoculation success
2. Factors in soil that limit successful BNF
3. Factors in the plant that limit nodulation and BNF

The following is a summary of program activities through April, 1984, taken from the FY 1983 Annual Project Report by Project Manager Dr. G. H. Elkan.

Grants awarded	63
U.S. scientists involved	45
Institutions involved (U.S.)	25
Projects currently active	40
Average funding/project	$57,000/3 years (approx. 50% for overseas collaboration)
LDC countries involved	31
LDC collaborators involved	49
Publications	58
Graduate students	31 (completed or in progress)

Present Situation

At this point, I don't know what the situation is with the BNF-LF grant program. However, a number of relevant questions have occurred to me, or have been suggested by others, which may be illuminating when answered.

1. What is the long-term benefit to the co-operating LDC in terms of permanent facilities, trained personnel, and solution of BNF problems?
2. What has been the nature of the follow-up? Is there evidence of maintenance of professional contact between U.S. and LDC scientists following termination of a grant? How often?
3. In broad terms, the objective of the grant program, from its inception to this date, has been to identify/study factors limiting BNF in LDC's. The term "limiting factors" is a very general term covering a multitude of sins. How often do we identify/study factors which are MOST limiting in a specific LDC situation? Are we studying factors which are uniquely limiting in the given situation or factors which have been generally recognized and extensively studied throughout the world? In designing these initial studies, are our priorities valid? As a hypothetical example, would it make sense to study the effects of seed-applied fungicide when the status of the indigenous rhizobial population is unknown or evidence indicates high levels of soil aluminum? Many limiting factors (such as pH, moisture, soil nutrient status, etc.) occur widely, are well-studied and readily recognized. In many

cases, minimal time, money and effort should be required to identify them in a specific situation.

This brings me to the crux of the matter. Is the general objective of the program - identification/study or limiting factors - really addressing the main issue?

I suggest that a more appropriate general objective of such a long-term program might be stated as that of making legume inoculation work in LDC's.

The process of achieving such an objective is certain to be very complex and would necessitate serious, long-term commitment on the part of U.S. and LDC scientists and the funding agency. Serious workers willing to commit themselves to a comprehensive long-term program should, once funded, not be subjected to abrupt curtailment of funds in the short term except under special circumstances.

Succinctly stated, I think we are dabbling part-time in a problem which merits our full-time, long-term attention as a critical aspect of our international agriculture policy.

Future Outlook

It is generally considered unhealthy, or at least ill-advised, to be critical of past actions without proposing improvements. I would

therefore like to propose an alternate scenario for a BNF-LDC program which differs markedly from the one now in place and which I believe would be an improvement. This may come at an auspicious moment since rumor is afloat that the current LF program may be terminated (and replaced with?) or altered to unrecognizable form(?).

I would like to begin with the stated assumption that the majority of factors significantly limiting BNF in LDC's are already known or at least readily identifiable. The general objective of a new or revised BNF program for LDC's then becomes that of making legume inoculation work by technology transfer. This is easier said than done. To begin with, the objective will not be achieved without the close cooperation of research, training and extension functions. The role of each would be as follows.

1. Research - to identify site-specific limiting factors, which may be "traditional" or unique to the locale; to adapt existing technology (or develop new technology) appropriate to the solution of identified problems.

   It is of utmost importance to choose carefully the technology to be transferred. What is the nature of the user group - small farmers or large farmers? Users with "high input" potential, capability and desire will not benefit from "low input" technology and vice versa. The technology to be transferred MUST be technologically, physically, and financially feasible for the target users.

Inherent in this should be the requirement that the US scientist initially spend a reasonable period of time (1 week minimum?) on-site with an LDC counterpart for purposes of problem-identification (in the field) and project planning. This should be a thorough effort, including analysis of the existing capabilities, potential and needs of the LDC infra-structure and the ability of the US worker to interface with those needs in research, training and extension. The research aspect is critical in identifying the right problems and choosing the appropriate technology.

2. Training - Training is critical as a guarantee of long-term, on-site continuation of the work (solutions evolve over a period of years, as a result of working experience). All personnel (field labor, graduate students, technicians, etc.) should be rigorously screened and only the best qualified selected into the program. It follows that LDC nationals would have preference as they might be more likely to remain. Better than "minimal" or "average" GRE scores and command of the english language should be a rigid requirement for graduate students in particular because of the large investment of time and money and the potential long-term gain or loss to the LDC program as a result of the quality of the final product. The research/training of students/technicians should have a strong orientation toward practical application of *Rhizobium*-legume technology, at the level appropriate to the country's needs. As an extreme example, it would do no good for the program to

train a Ph.D. in electron microscopy when a country needs to isolate and screen strains of rhizobia (and the country won't have EM available)!

A very important facet of the training process is the development of attitude. Often, and in many ways, we develop in students the idea that basic research using the latest technology (ex. genetic engineering) is the only way to go. It is frustrating to witness the attitude that applied research (field tests) using "old" methods (such as Kjeldahh N analysis vs acetylene reduction) is considered a degrading level of science. To the contrary, done properly, it is excellent science, it is at a level which is generally feasible in LDC's, and it is what is needed most in LDC's.

3. Extension - Extension is perhaps the most common "limiting factor" in any such BNF program, because it is generally absent or ineffective in all LDC's. If an extension service is absent and the LDC is not willing/able to initiate such a service, then it is incumbent on the scientists (US and LDC) to make provision for this function in formulating their plan of work or program design. It makes no sense to do site-specific work if it is not made available to the farmer.

Whenever possible, the project should have "small farm" orientation. LDC's are generally characterized by large populations of subsistence farmers with low agricultural production. They must be kept employed, but with increased

production capability. They have minimal education and capital, therefore the technology to be adapted and adopted must be SIMPLE and INEXPENSIVE. An effective extension mechanism may be emphasis of on-farm trials with "successful" or progressive" subsistence farmers.

SUMMARY:

Analysis of Production System

↓ 1

Limiting Factors (other than BNF)

↓ 2

Legume + Fertilizer N = Good Production

↓ 3

Legume-Fertilizer + *Rhizobium* (Successful BNF)

↑ 4

Rhizobium-Legume Technology (the END - not the BEGINNING)

LIMITING FACTORS PROGRAM QUESTIONNAIRE

Please check or fill in the blanks as appropriate. Please do not sign. We ask you to be frank. This information will not only be used to evaluate the present program but also to help in future program development.

I am a U.S. Co P.I. _____
non U.S. Co P.I. _____

My Limiting Factors grant has expired
is ongoing
is new (1984 or 1985)

This is (was) my first overseas project Yes No

This is (was) the first time I collaborated with a U.S. scientist
Yes No

I worked with my Co P.I. prior to submitting a joint proposal to the Limiting Factors program. Yes_____ No_____

If yes, how many years? _____

Although my Limiting Factors Project has terminated, I still collaborate with my co-PI from the LF grant. Yes_____ No_____ Not Applicable _____

Problems getting a Memorandum of Understanding signed delayed the start of the project. Yes_____ No_____

There were problems coordinating activities. Yes_____ No_____

Communication problems were : nonexistent _____
occasional _____
severe _____

Improved communication might be achieved by : _____
_____
_____

Timely disbursement of funds was a problem Yes _____ No _____

The funds were fairly budgeted for tasks to be accomplished.
Yes_____ No _____
If no, please explain what would have solved this problem._____
_____
_____

Program management helped me solve probelms related to the project
Yes _____ No. _____
If yes, please state what problems program management helped you solve.
_____
_____

My Co P.I. visited me too often _____ not often enough_____
as was appropriate_____

Visits were too short _____ too long _____ ok _____ duration.

I wrote part of the original project proposal Yes _____ No _____

I conferred once _____ several times _____ many times _____ with my Co P.I. during the preparation of the proposal.

I contributed significantly to project design. Yes _____ No _____

My Co P.I. accomplished his work plan. Yes _____ No _____

Personality incompatabilities made it difficult to meet project goals.
Yes _____ No _____

I acquired new skills/ expertise from my Co P.I. Yes _____ No _____
Please explain _____

My institute benefited from my collaboration withmy co-PI. Yes _____ No _____
If yes, please explain how _____

The most serious problem I encountered working on the project is (was):
_____

The greatest benefit I derrived from collaboration on this project is (was): _____

I believe collaborative programs of this type are valuable _____
not valuable _____
If valuable, please explain why. _____

If not valuable please explain why _____

Have the coordinational meetings for the Limiting Factors Program been frequent enough: yes _____ no _____ (Note that it costs as much to hold one coordinational meeting as to fund one proposal).

Have you had a visit from either the Limiting Factors project manager, a USDA official, or the AID Project Officer during your grant?
yes _____ no _____

If you had a visit was it useful in solving any LF project problems?
yes _____ no _____

If you did not have a visit would you like (have liked) one?
yes _____ no _____

Please add below any suggestions you may have for changing either the management design, implimentation, operational framework, or goals of the Limiting Factors Program.

THANK YOU FOR YOUR CANDID RESPONSES.

QUESTIONAIRE

Working Group I- Plant factors that limit BNF.

Members:

J.C. Miller. Chairperson
J.C. Rosas, Rapporteur

J. Wynne
R. Abilay
D. Baltensperger
V. Saka
S. Zuluaga
T. Schneeweis
D. Prahdam
J. Fernandez

Objective: The goal of this working group is to address the issues listed below and develop a consensus among the group which will be presented by the rapporteur. Although the development of a consensus may be difficult for some issues, it is the output being sought.

The call for proposals of the Limiting Factors Program lists 4 plant factors that limit nodulation and BNF:

a. Genetic capability of the plant-rhizobia system for BNF
b. Deficiencies and toxicities of chemical nutrient elements
c. Production potential of the plant
d. Plant pests and diseases

1. Which factor from the list above or from this work group's studies is the most important plant factor that limits BNF?

2. For which species is this factor most important?

3. Under what environmental conditions is this factor most important?

4. What other factors are also important, please list in descending order of importance.

5. What plant limiting factor was addressed most frequently in the Limiting Factors grants held by members of the group?

6. If the factor in question 5 was not the same as that listed in question 1 why was the factor in question 5 studied so often?

7. Can molecular biology eliminate the plant constraining factor listed in question 1?

8. Explain how?

9. Can the development of promiscuous nodulating plant lines eliminate the limiting factor listed in question 1?

10. Explain how?

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11. Did you use the plant cultivars recommended at the 1981 Limiting Factors meeting in Washington D.C. in your projects? Please indicate number yes vs no.

12. For those who answered no, why not?

13. Did you use the rhizobial strains recommended at the 1981 Limiting Factors meeting in Washington D.C. Please indicate number yes vs no.

14. For those who answered no, why not?

15. Can greater gains in BNF be expected by removing the plant limiting factors than by removing other limiting factors, i.e. soil stresses, ineffective rhizobia etc.?

16. If the answer to 15 is yes please explain why.

17. Should a larger percentage of the Limiting Factors grants be awarded to projects addressing plant limiting factors, or is the present blend ideal?

18. Develope a strategy for elimating the limiting factor listed in question 1.

19. If the factor listed in question 1 should be eliminated as a result of the strategy devised in question 9, what would be next most serious plant constraint to BNF?

20. If the Limiting Factors program were to be severely cut in funds which of the five areas should be eliminated:

Strain selection and inoculation response studies
Plant factors that limit BNF
Soil factors that limit BNF
Rhizobial factors that limit BNF
Constraints to implimentation of inoculant technology

21. Of the four topics that remain, please rank them in order of importance.

22. If it were necessary to drastically cut the program which of the five topics should continue to receive funding.

23. Is there another topic that should be addressed as a high priority other than the five listed above.

24. What should be the scientific goal of the Limiting Factors program?

QUESTIONAIRE

Working Group II. Strain selection and response to inoculation

Members:

D. Bezdicek, Chairperson
I. Pepper, Rapporteur

E. Pardo	J. Elkan
R. Graham	D. Johnson
C. van Kessel	F. Dazzo
A. Sexstone	E. Schroeder
Z. Shamsuddin	Y. Velasquez

Objective: The goal of this work group is to address the issues presented below and develop a group consensus to be presented by the rapporteur. While it may be difficult to arrive at a consensus for some of the issues this is the output being sought.

1. What generalizations about response to inoculation can be drawn from the studies done in the Limiting Factors Program?

2. From these studies can a positive response to inoculation be reliably predicted?

3. For what species can we predict a response to inoculation?

4. What was the most common experimental design employed in the response to inoculation trials?

5. Were the rhizobia strains recommended at the Washington D.C. 1981 Limiting Factors meetings employed in the inoculation reponse studies? Please indicate number of studies that did and didn't.

6. If the recommended strains were not used in some studies, why not?

7. Were the plant cultivars recommended at the Washington D.C. 1981 Limiting Factors meeting used in the studies? Please indicate number yes and no.

8. If the standard strains were not used in some of the studies why not?

9. Should more inoculation response studies be conducted as part of the Limiting Factors Program?

10. Explain the answer given for 10.

11. If the answer to question 10 was yes, should the studies be focused on one species, soil type, etc. or kept broad?

12. What is the major constraint to obtaining a response to inoculation in the field?

13. With which crops is the constraint in question 12 most serious?

14. Can molecular biology be employed to overcome the constraint in question 12?

15. If yes please explain how?

16. If the answer to question 12 is no, what strategy should be used to oversome this constraint?

17. Is the development (breeding) of promiscuous nodulating plant lines the ultimate solution to obtaining reliable nodulation in LDCs?

18. Design a program, strategy, or experimental approach that would lead to the ability to predict incoualtion response.

19. Devise a strategy to assure the LDC farmer a positive response to inoculation.

20. If the Limiting Factors program were to be severely cut in funds which of the five areas should be eliminated:

Strain selection and inoculation response studies
Plant factors that limit BNF
Soil factors that limit BNF
Rhizobial factors that limit BNF
Constraints to implimentation of inoculant technology

21. Of the four topics that remain, please rank them in order of importance.

22. If it were necessary to drastically cut the program which of the five topics should continue to receive funding.

23. Is there another topic that should be addressed as a high priority other than the five listed above.

24. What should be the scientific goal of the Limiting Factors program?

QUESTIONAIRE

Working Group III. Soil factors that limit BNF

Members:

P. Graham, Chairperson
R. Lockerman, Rapporteur

S. Abdel-Ghaffar
D. Hubbell
J. Sartain
W. Lindeman
T. Shilling
C.E. Salazar
H. Skipper
L. Wamocho

Objective: The goal of this working group is to address the issues presented below and develop a group consensus to be reported by the rapporteur. While it be difficult to come to a consensus on some of the issues, this is the output being sought.

The call for proposals for the Limiting Factos Program lists the following soil factors that may limit BNF:

a. ineffective rhizobial strains
b. soil temperature and soil moisture
c. acidity, alkalinity, and salinity
d. nutrient deficiencies
e. antagonistic effects of plant roots
f. antagonistic effects of other soil microorganisms
g. pesticides and fertilizers

1. What factor from the list above or from this work group's studies would you consider the most important soil limiting factor?

2. What soil limiting factor was the one most commonly addressed by this group's Limiting Factors grants?

3. If the factor in question 2 is not the same as question 1, why was the factor in question 2 chosen so often?

4. What generalization about the factor in question 2 can be drawn from the projects?

5. Did the projects identify a strategy for overcoming the negative effects of the most limiting soil factor?

6. Explain the strategy.

7. Were the rhizobia strains recommended at the Washington D.C. 1981 Limiting Factors meeting used in the studies? Please indicate the number of studies that did and didn't.

8. If the standard strains were not used in some studies, why not?

9. Were the plant cultivars recommended at the Washington D.C. 1981 Limiting Factors meeting used in the studies? Please indicate the number of studies that did and didn't.

10. If the recommended cultivars were not used in some studies, why not?

11. Should the Limiting Factors program concentrate more on soil limiting factors?

12. If the answer to 11 is yes, why?

13. What other soil limiting factors are also serious constraints to BNF?

14. Have any of the projects found a successful way to overcome these constraints?

15. If the answer to question 14 is yes, please explain how?

16. Was the search for mechanisms to oversome soil limiting factors the major thrust of the studies?

17. Can molecular biology play a role in eliminating the major soil limiting factors?

18. If the answer to 17 is yes please explain how.

19. Develop a strategy, program, or series of experiments that would led to a mechanisms to overcome the main soil factor limiting BNF listed in question 1.

20. If the Limiting Factors program were to be severely cut in funds which of the five areas should be eliminated:

Strain selection and inoculation response studies
Plant factors that limit BNF
Soil factors that limit BNF
Rhizobial factors that limit BNF
Constraints to implimentation of inoculant technology

21. Of the four topics that remain, please rank them in order of importance.

22. If it were necessary to drastically cut the program which of the five topics should continue to receive funding.

23. Is there another topic that should be addressed as a high priority other than the five listed above.

24. What should be the scientific goal of the Limiting Factors program?

QUESTIONAIRE

Working Group IV. Rhizobial factors (including inoculants) that limit BNF.

Members:

R. Weaver, Chairperson
S. Smith, Rapporteur

B. Hernandez
A. Trotman
T. Schneeweis
N. Boonkerd
C. Ramirez
P. Wadisirisuk
C. Vidor
L. Cakmakci
A. Wollum
J. Burton

Objective: The goal of this work group is to address the issues presented below And develop a group consensus to be presented by the rapporteur. While it be difficult to arrive at a consensus for some of the issues, this is the output being sought.

1. What is the major constraint to successful implimentation of inoculant technology in LDCs?

2. Does the outcome of the projects suggest a possible solution to this constraint? What is it?

3. What are secondary constraints to use of inoculant technology in LDCs.

4. Which of these should be addressed should the major constraint be resolved?

5. Develop a strategy, program, or conceptual approach for overcoming the major constraints to the use of inoculant technology in developing nations.

6. Should more emphasis in the Limiting Factors Program be placed on factors limiting inoculant quality and inoculation success?

7. If all the Limiting Factors resources were focused on the resolving the problems of inoculant technology and a solution were found, what percent yield increaes could be expected?

8. Is competition the major rhizobial constraint to increasing BNF?

9. If no, what characteristic of rhizobia is more important in this regard?

10. Do the results of the projects suggest a method for overcoming the negative effects of competition? What is it?

11. If the projects do not suggest a method, can the group members suggest a strategy for overcoming the negative effects of competition? Please explain.

12. Should more emphasis be placed on competition in the Limiting Factors program?

13. If all Limiting Factors resources were dedicated to resolving the problems of competition, and an actal solution were found, hypothesize what percentage increase in yield might be expected?

14. Is competition in inoculants a theme that should be investigated?

15. Were the rhizobial strains recommended at the Limiting Factors meeting in Washington D.C. in 1981 used in the competition studies. Please indicate number of studies that did and did not.

16. If the recommended strains were not used, please indicate why not.

17. Does molecular biology offer a solution to the problem of competition?

18. Is it the most promising avenue for success?

19. Would the development of promiscuous nodulating plant lines for all species remove competition as a constraint to BNF? Please explain.

20. If the Limiting Factors program were to be severely cut in funds which of the five areas should be eliminated:

Strain selection and inoculation response studies
Plant factors that limit BNF
Soil factors that limit BNF
Rhizobial factors that limit BNF
Constraints to implimentation of inoculant technology

21. Of the four topics that remain, please rank them in order of importance.

22. If it were necessary to drastically cut the program which of the five topics should continue to receive funding.

23. Is there another topic that should be addressed as a high priority other than the five listed above.

24. What should be the scientific goal of the Limiting Factors program?

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PROCEEDINGS OF
COORDINATED WORKSHOP
BNF LIMITING FACTORS PROGRAM
WASHINGTON, D.C. FEBRUARY 22-23, 1982

Introduction

The Workshop was designed to bring together the principal investigators funded under the USDA-CSRS-AID program titled "Symbiotic Nitrogen Fixation: Studies of Factors Limiting N-Fixation for LDC Crop Production". In place since 1976, this project has involved some 35 research scientists at 22 U.S. universities and experiment stations. Though two earlier meetings in connection with this program had been held, this was the first attempt to bring all active researchers together at one time.

The Workshop was held in Washington, DC on February 22-23, 1982. It was organized to address three areas of major concern. Briefly, these were:

1. To identify and document the most effective and competitive strains of *Rhizobium* spp. for the major flood, forage and tree legumes;	Work Group A
2. To identify and document those varieties and/or selections of major agricultural legumes best adapted to tropical soils; and	Work Group B
3. To document the unique and valuable experiences of the Principal Investigators that have occurred as a result of cooperative agreements with scientists, institutions and agencies within emerging countries.	Work Group C

Participants at the Workshop included the Program officials, past and present Principal Investigators, and several guests from both NSF and AID.

A list of participants is provided in Appendix A.

Report of Work Group A

Work Group Chairman - David Bezdicek - Washington State University

The following criteria were agreed upon as a basis for selecting the strains of *Rhizobium* spp. to meet Objective 1.

a. Strain shown to be effective and/or highly competitive in greenhouse and preferably field trials.

b. "Serologically distinct".

c. Stability in culture and adaptability to certain environmental conditions.

d. Availability of supporting data in the literature.

The following represent those cultivars and associated *Rhizobium* strains that were discussed and selected as most effective.

Cultivar	Strains selected	Source
Beans (*Phaseolus vulgaris*)	Kim-5* (highly competitive)	Washington State Univ.
	C-05 (CIAT)	NifTAL
	TAL-182	NifTAL
	127K17	Nitragin
Cowpeas (*Vigna* spp.)	32H-1*	Nitragin
	TAL-169 (acid tolerant)	Beltsville
	THA-201 (good survival)	Texas
Peanuts (*Arachis hypogaea*)	32H-1*	Nitragin
	TAL-169	NifTAL
	T_1 (TAL 1371)	NifTAL
	TAL-1000	NifTAL
	176A34	Nitragin
Pidgeon pea (*Cajanus cajan*)	32H-1*	Nitragin
	IHP155	ICRASAT (Schroeder, Puerto Rico)
	IHP147	California (Focht)
Mung bean (*Vigna radiata*)	TAL-169(176A22)*	NifTAL
	THA-301	NifTAL
	M_6	NifTAL
Chickpeas (*Cicer arietinum*)	27A3*	Nitragin
	27A8	Nitragin
Peas (*Pisum sativium*)	N92A3*	Nitragin
	C1204	Washington State Univ.
	128A12	Nitragin
	128C53	Nitragin
Lentils (*Lens culinaris*)	N92A3*	Nitragin
	I11 (Hawaii 5-0)	NifTAL
	128A12	Nitragin
Fababeans (*Vicia faba*)	175 F12*	Nitragin
	175F10	Nitragin
Soybeans (*Glycine max* L.)	110*	Beltsville
	122	Beltsville
	138	Beltsville
	142	Beltsville
	143	Beltsville
	6	Beltsville
Lima beans (*Phaseolus linensis*)	?	Beltsville
Winged beans (*Psophocarpus tetragonolobus*)	?	NifTAL
Lupine (*Lupinus albus*)	?	NifTAL and
Lupine (*Lupinus angustifolius*)	?	Beltsville
Other seed legumes	?	NifTAL, Beltsville
Alfalfa (*Medicago sativa*)	?	Beltsville
Other Forage Legumes	?	?
Tree Legumes	?	NifTAL
Centrosema		
Stylosanthus		
Desmodium		

*Considered the standard of reference. All strains are considered to be effective, although the one listed first should always be included in comparative trials.

The lack of information for those legumes listed last occurred because of time constraints for further discussion and because few of the workshop participants were working on these species.

This list of strains is certainly not the final word and they are listed to be evaluated and challenged. Hopefully, these selections will find utility as standard strains for comparison to other new isolates in both laboratory, greenhouse and field studies. This list should also prove valuable for scientists beginning research work on BNF.

Finally, antisera is available for many of the strains listed but excess will have to be negotiated on a case-by-case basis from the person or agency identified with each strain.

In general, the objectives of Working Group A were met during this meeting. It is hoped that we can continue to communicate on the standard strains and exchange new data on additional strains. This may provide a justification for another meeting in the future. Some additional topics may include strategies in breeding for enhanced N_2 fixation and techniques for measuring N_2 fixation in the field. The latter topic is important in arriving at a N budget, especially in developing countries where modern techniques are not available. We should consider non-nodulating legumes, ineffectively nodulated legumes and even non-legumes when we are attempting to estimate N_2 fixation by difference in total plant nitrogen. Here again, the plant breeders may be able to provide input.

Report of Work Group B

Work Group Chairman - Johnny Wynne - North Carolina State University

Workgroup B spent considerable time discussing the following topics:

1) Factors affecting BNF by the macrosymbiont

The discussion of factors that may limit symbiotic fixation revealed little that was startling. However, there appears to be some evidence that the absence of mycorrhizae may limit nodulation under desert conditions.

The discussion raised two ancillary questions. One related to the response of well-nodulated legumes to fertilizer nitrogen. Is this an indication of insufficient fixation? The consensus was that virtually all annual legumes seem to respond to fertilizer N, even when nodulated; on the other hand, most perennials do not. For some, such as *V. faba* and mung, little information is available.

The other question revolved around the possibility of looking at the coevolution of host species and the rhizobial symbiont, as compared to those host species that may have evolved under conditions of high native soil-nitrogen. The conclusion seemed to be that such an effort would yield little worthwhile information, on one hand because of the paucity of good archeological informaton and, on the other, because of known variability in response within species, or genera, for which we

have "known" centers of origin. For example, all the wild species of limas, P. vulgaris and, perhaps, cowpeas appear to be perennials.

2. Methods for measuring BNF in legume symbioses

The next question that arose was, "What is it we are trying to measure, and how should we do it?" It was agreed that, for most pulse species, there is little correlation between grain yield and nitrogen-fixation efficiency. Therefore, some attempt must be made to evaluate symbiotic fixation per se. In ascending order of sophistication (and cost?), and assuming the crop is nodulated, estimates may be obtained by:

Recording nodule weight and color
Measuring Kjeldahl nitrogen
Analyzing by Acetylene reduction
Using Xylem-sap analysis (ureides)
Using N_{15} measurements

Each of these methods has its limitations; concern was expressed that these are too rarely considered. It was generally agreed that grain yield is the ultimate criterion but that, given reasonable yields, other variables must be measured. There was some discussion about the use of yield as such as the ultimate measure; some argument can be made for yield stability as being the more desirable objective.

3. Breeding strategies for enhancing BNF

Regarding breeding strategies for enhancing BNF it was generally agreed that measurements within individual genotypes in early generations are futile. For most crop species, reliable data and reasonable C.V.'s can only be obtained by evaluating pure or near pure lines in replicated trials at multiple locations. The feeling was that, from a breeding standpoint, nitrogen-fixing capacity (the efficiency of the host-strain interaction) is a quantitative trait similar to yield. Therefore, the selection and breeding strategies are comparable; there are no valid shortcuts. The breeders felt that their appropriate role is to create elite germplasm (lines) that have all the desirable characteristics of yield, disease resistance, etc., plus the ability to support a high level of N fixation, and then to use them or to release them for use as parents for local breeding program.

4. Identification of varieties, cultivars, and/or selections of major agricultural legumes as standards for breeding programs

Regarding the identification of specific genotypes that might be used as standards against which progress could be measured, the group was very reluctant to be too specific. Their feeling was that there is no single standard available for any crop species. The best approach, particularly for food legumes for which dietary preferences and taboos are usually strong, is to use the best local cultivars (agrotypes?) as the standard. If the area in question can be characterized climatolo-

gically and edaphologically, any competent U.S. breeder should be able to suggest a list of cvs. or P.I.'s that might be compared with the local standards. An alternative is to make a list for those species for which we have sufficient information from which the local investigator might choose. The main purpose such a list would serve would be to reduce the decisions a researcher would have to make. This is certainly a less desirable alternative than tailor-making a specific list for an identified site. Some tentative lists are below.

MUNGBEAN - Suggested genotypes from Asian Vegetable Research Development Center, Taiwan.
High nitrogen-fixing potential 2184
Low nitrogen-fixing potential 1484

PEANUTS*- Peanut cultivars suggested for:

High Nitrogen Fixation and Nodulation

Cultivar	Botanical Variety	Test Site
Florigiant	hypogaea	Texas, North Carolina, U.S.A.
Va 72R	hypogaea	North Carolina, U.S.A.
NC 5	hypogaea	North Carolina, U.S.A.
NC 6	hypogaea	North Carolina, U.S.A.
NC 4	hypogaea	North Carolina, U.S.A.
Florunner	hypogaea	Texas, North Carolina, U.S.A.
Robut 33-1	hypogaea	North Carolina, India
X-14-4-B-19-B (ICG 1561)		India
NCAcc 2821 (ICG 2405)	hypogaea	India, North Carolina

Acid tolerance

UF 78305	hypogaea	
UF 78307	hypogaea	

For selection of Rhizobium strains with broad adaptation to the host (lack of host specificity), strains should be tested with and perform well with diverse germplasm such as:

Florigiant - Virginia
NC 6
Argentine
Tamment
Tennessee Red
New Mexico Valencia - Valencia

*Contact Dr. Johnny Wynne, North Carolina State University

COWPEAS*

Screened for salt tolerance*. Inoculated with CB-756, granular.
Soil N (ppm) 5.05 - 22.80. Arizona.
Soluble salts (ppm) 5,327 - 29,407

	Ranking with respect to:		
	Yield	Nodule #	% Noduled Plants
P.I. 211642	1	3	4
353011	2	1	1
353380	3	2	2
180494	4	6	3
353332	5	7	7
293518	6	8	8
Ca. #5	7	5	6
Speckled Purple Hull	3*	4	5
*not mature			

Suggested Texas genotypes**. High nitrogen-fixing potential.

108	187	3795
113	1630	3837
129	2408	3848
174	3764	3851

Suggested P.I.'s

P.I.	180358	354861
	293458	382088
	354481	382115
	354858	

Commercial Checks.

Moderately high fixer - Brown Crowder
Moderately low fixer - Bush purplehull

*Contact Dr. Victoria Macarian, University of Arizona
**Contact Dr. J. Creighton Miller, Texas A & M University

Recommended lines of bean (phaseolus vulgaris L.) for biological nitrogen fixation experiments.*

Class	Line	BNF Potential[z]	Source[y]
White	1. Sanilac	L	1
	2. Seafarer		1
	3. Swan Valley		1
	4. Neptune		1
	5. C-20		1
	6. Fabico 23	I	1,3
	7. Bunsi		1
	8. Aurora		1,8
	9. BAT 340		5
	10. BAT 1061	I	5
	11. BAT 1280		5
	12. UW 24-21	H	3
	13. UW 24-55	H	3
	14. UW 24-5	H	3
	15. UW 24-65	H	3
Black	1. Black Turtle Soup	I	3
	2. T-39		1
	3. Midnite	L	1
	4. Domino		1
	5. Black Magic		1
	6. Puebla 152 Black	H	3,5
	7. Jamapa	I	3,5
	8. Porrillo Sintético	H	3,5
	9. ICA Pijao	H	3,5
	10. UW 20-46	H	3
	11. UW 20-55	H	3
	12. UW 21-58	H	3
	13. UW 21-43	H	3
	14. UW 21-19	H	3
	15. BAT 76	I	3,5
	16. BAT 1320	L	5
	17. BAT 304	L	5
Pinto	1. UI-111	L	7
	2. UI-114		7
	3. Olathe		7
	4. Pindak		7
	5. NW 590		4
	6. NW 410		4
Great Northern	1. UI-59	L	6
	2. Valley		6
	3. GN 1140		6

Class			BNF Potential	Source
Red Mexican	1.	Rufus	L	4
	2.	Big Bend		7
	3.	UI-36		4
	4.	NW 59		4
	5.	NW 63		4
Pink	1.	Viva	I	4
	2.	Sutter Pink		4
	3.	Rosa		4
	4.	A159		5
	5.	6R-1053		4
Red	1.	Montcalm	L	1
	2.	Charlevoix	L	1
	3.	Sacramento LRK		2
	4.	California LRK		8
	5.	California DRK		8
	6.	Redkloud		9
	7.	BAT 1249		5
	8.	BAT 1274		5
Snap	1.	BBL 240	L	3
	2.	Harvester		11
	3.	Cascade		11
	4.	Sprite		11
	5.	Triumph		11
	6.	Lake Superior		11
	7.	Astro		10
	8.	Eagle		10
	9.	Spurt		10
	10.	BBL 274		10
	11.	Goldrush		10
	12.	Kentucky Wonder Pole		10

[z]BNF potential as measured by acetylene reduction assay in Wisconsin. H = high, I = intermediate, L = low

[y]See Appendix B

*The list of recommended lines of P. vulgaris have been divided into 8 categories based on the commercial classes of most importance in the U.S.A. and represents either widely-grown and well-adapted cultivars or experimental lines of exceptional promise. Some of the lines have been characterized for BNF and most have shown excellent yield potential in the U.S.A. Yield and BNF will vary with experimental conditions. The recommended lines vary widely for many traits; questions concerning the characteristics and suitability of a specific line for a given region or set of experimental conditions may be answered by the plant breeders listed under Sources or other BNF workers. See Appendix B.

5. Future trends and goals for improved BNF through Plant Breeding

The discussion of future trends and goals for improved BNF through plant breeding was wide-ranging. It was agreed that BNF efficiency was pretty low on everyone's list of priorities. However, after other attributes such as disease resistance are accounted for, nitrogen-fixing capacity may come into play. No breeder in the room actually was selecting with BNF in mind; for some crops such selection may be fortuitous, particuarly if no N fertilizer is used on the breeding plots.

Questions that should be addressed are, "What effect does N fixation have on yield?" In peanuts, for example, it appears that 25% of the variation in yield was related to BNF. What effect does this have on associated crops in interplanting or on subsequent crops in the relay? Another point that requires more study is, "What plant traits (that might be more easily quantified) are associated with high N fixation capacity?" Again, with peanuts, high leaf weight, leaf area, or leaf area duration seem to be strongly correlated with high BNF.

We need to know more about the N-response curves of tree legumes. We need more support for Rhizobium, as well as plant, collections. We should not over-emphasize the utility of non-nodulating lines. We should be more careful to publish the plant germplasm, as well as the bacterial strains, used in our studies.

It was also suggested that we should be looking at patterns of plant growth and development with respect to their susceptibility to manipulation (management, as well as breeding) in support of higher nitrogen fixation. It was generally agreed that all components of BNF are genetically controlled and, therefore, heritable. Whether or not heritabilities are high enough to be reasonably exploitable remains to be seen. Under any circumstances, this is long-term work. Three years would represent just a beginning of such a program. Funding agencies must keep this fact in mind.

Finally, it was agreed that, speaking of most crop species, generalizations may cause more time to be wasted than would be invested in research to answer the specific question raised.

6. Miscellaneous discussions - Groups A & B

A discussion followed for both working Groups A and B which centered around strategies for the microbiologist and plant breeder to enhance N_2 fixation in legumes. Some questions and comments are as follows:

1. In genotype selection for enhanced N_2 fixation, should a single strain or multiple strains be used? One comment was that it is difficult to do both the genotype and strain evaluation adequately at the same time. One suggestion was to use a mixture of strains for the initial genotype evaluation

and then look for specific host-strain combinations in a later evaluation. However, we eventually run into the old problem of the added strains not being able to compete with indigenous ones.

2. Some discussion centered around using a high nitrogen control in greenhouse studies. One suggestion was to compare total plant growth and/or total plant nitrogen of the inoculated genotype to that of the same genotype under adequate nitrogen. This would seem to be a better approach than to compare the inoculated plant to the uninoculated control.

3. Some concern was expressed on the level of available soil nitrogen present in many plots used in the routine evaluation of new selections in legume breeding programs. In plots where legumes are planted continuously and not rotated with non-legume crops, soil nitrogen may build up. We should not expect to see a non-efficient host-strain combination to be expressed or identified if soil nitrogen levels are too high. It is therefore possible to carry along genotypes in a breeding program that are rather inefficient in N_2 fixation simply because of high levels of soil N. This is especially important in the LDC's where soil nitrogen may be low and even in the U.S. where costs of fertilizer N of $500/ton are estimated in the next three to four years.

4. There was some discussion on the selection of a few genotypes of each species to be used as standards by soil microbiologists in strain evaluation. The plant breeders generally agreed that is is not possible to pick only several "standards" of genotypes. This is understandable in field studies where genotype adaptability is related to so many environmental and disease related factors. However, in greenhouse studies, it should be possible to select one or several genotypes the soil microbiologist can use.

Report of Workgroup C

Workgroup Group C was made up of all the workshop participants. This workshop had as its main objective the sharing of experiences with cooperative research in LDC countries which might be of benefit to other participants. Presentations were made by Dr. Bill Judy, Africa Bureau; Dr. Mary Clutter, National Science Foundation; and Charles Smith, USDA-CSRS.

Topics discussed by the particpants included problems in providing appropriate training experiences for foreign graduate students; conducting research programs in LDC countries, communication of research results between scientists; and problems of transferring research grant dollars to LDC cooperating scientists.

Appendix A

List of Workshop Participants

Participant	Institution
D. F. Bezdicek	Washington State University
B. Ben Bohlool	University of Hawaii
William Bryan	West Virginia University
G. H. Elkan	N.C. State University
Dennis Focht	University of California, Riverside
H. D. Gross	N.C. State University
Dave Hubbell	University of Florida
William Lindemann	N. Mexico State University
Ron Lockermann	Montana State University
Thomas Loynachan	Iowa State University
James R. McFerson	University of Wisconsin
Victoria Macarian	University of Arizona
J. Creighton Miller	Texas A & M
Ian Pepper	University of Arizona
Harold L. Peterson	Mississippi State
Don Phillips	University of California, Davis
Walt Scudder	University of Florida
Larry Shuman	University of Georgia,(Exp. Station,GA)
Jim Sims	Montana State University
Carl Tucker	University of Califonria, Davis
Richard Weaver	Texas A & M
Barbara Webster	University of California, Davis
Davis O. Wilson	University of Georgia, (Exp. Station,GA
Arthur Wollum	N.C. state University
Sarah Wright	West Virginia University
Johnny C. Wynne	N.C. State University
Jake Halliday	NifTAL, University of Hawaii
Deane Weber	USDA/ARS, Beltsville
Harold Keyser	USDA/ARS, Beltsville
Joe Burton	Nitragin Co. Milwaukee
Richard Graham	Univ. West Indies/Cornell University
Mohamid Hadad	Sudan/Iowa State University
Robert Miller	N.C. State University
Lloyd Frederick	US AID, Washington, DC
David Walker	Texas A & M

Appendix B

SOURCES: Seed and germplasm descriptions may be obtained from the following workers:

1. Dr. M. W. Adams
   Dept. of Crop and Soil Sciences
   Michigan State University
   East Lansing, MI 48823
   517/355-2234

2. Dr. Iver Johnson
   Sacramento Valley Milling
   Ordbend, CA 95943
   916/934-3385

3. Dr. F. A. Bliss
   Dept. of Horticulture
   Univ. of Wisconsin
   Madison, WI 53706
   608/262-1623

4. Dr. D. W. Burke
   USDA-ARS
   Irrigated Agriculture Research and Extension Center
   Prosser, WA 99350
   509/786-3454

5. Genetic Resources Unit
   Centro Internacional de Agricultura Tropical
   Apartado Aereo 6713
   Cali, Colombia S.A.

6. Dr. D. P. Coyne
   Dept. of Horticulture
   Univ. of Nebraska
   Lincoln, NB 68503
   402/472-1126

7. Dr. J. J. Kolar
   Univ. of Idaho Research and Extension Center
   Kimberly, ID 83341
   208/423-4646

8. Carl Tucker
   Dept. of Agronomy and Range Science
   University of California
   Davis, CA 95616
   961/752-6606

9. Dr. D. H. Wallace
   Dept. of Plant Breeding
   and Biometry
   Cornell University
   Ithaca, NY 14853

10. Dr. Dave Webster
    Asgrow Seed Company
    P. O. Box 290
    Filer, ID 83328
    208/326-4321

11. Dr. Keith Zary
    P. O. Box 20726
    Sun Seeds
    Farmington, MN 55024
    612/463-4646