ANNUAL REPORT
1984

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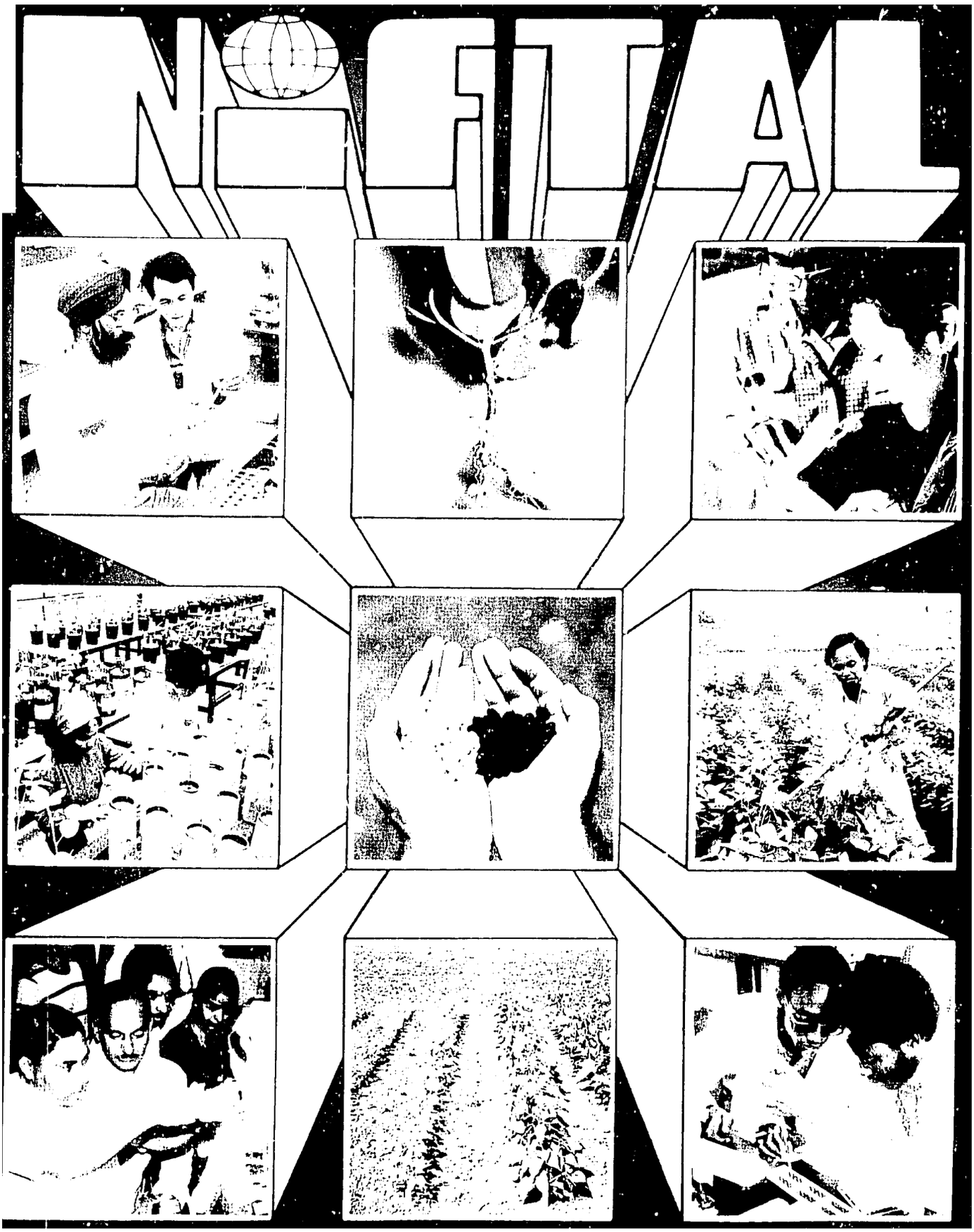

On the cover . . .

NIFTAL

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1. Senior legume scientists learn advanced techniques in NifTAL's intern program.

2. Legumes can be self-sufficient for nitrogen thanks to the rhizobia housed in their root-nodules.

3. A Honduran researcher is trained in antisera production at a NifTAL course in Mexico.

4. International scientists receiving NifTAL training on *Rhizobium* strain selection in Malaysia.

5. Inoculated bean seeds on the right have the edge—10,000 rhizobia/seed.

6. One of over 200 scientists participating in NifTAL's International Network of Legume Inoculation Trials.

7. A refresher course on Inoculant Production and Quality Control taught at Hyderabad, India.

8. Common beans *(Phaseolus vulgaris)* respond markedly to inoculation with acid tolerant rhizobia in an Ultisol.

9. African researchers learn how to test for successful inoculant strains at NifTAL's Nairobi course.

NITROGEN FIXATION BY TROPICAL AGRICULTURAL LEGUMES

NFTAL

University of Hawaii/USAID

ANNUAL REPORT 1984

USAID Contract No. DAN-0613-C-00-2064-00
01/01/83 - 12/31/84

Dr. Jake Halliday, Principal Investigator

Department of Agronomy and Soil Science
Hawaii Institute of Tropical Agriculture and Human Resources
College of Tropical Agriculture and Human Resources

UNIVERSITY OF HAWAII

STATISTICAL SUMMARY

Title	: Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL Project)
Contract Number	: Dan-0613-C-00-2064-00 (was AID/ta-C-1207)
Project Number	: 931-0613
Principal Investigator	: Jake Halliday (till November 15, 1984)
Address	: NifTAL Project and Mircen P. O. Box 0 Paia, Hawaii 96779 USA
Contractor	: University of Hawaii Department of Agronomy and Soil Science College of Tropical Agriculture and Human Resources 3050 Maile Way, Honolulu, HI 96822
Contract Period	: 06/16/82-12-31/86
Reporting Period	: 01/01/84-12/31/84
USAID Funds to Date	: $3,725,000
Current Year Expenditures	: $1,162,729
Estimated 1985 Funds	: $1,150,000
Total Contract Funds	: $4,875,000
Project Office	: S&T/AGR/RNR
Project Monitor	: Dr. Lloyd R. Frederick

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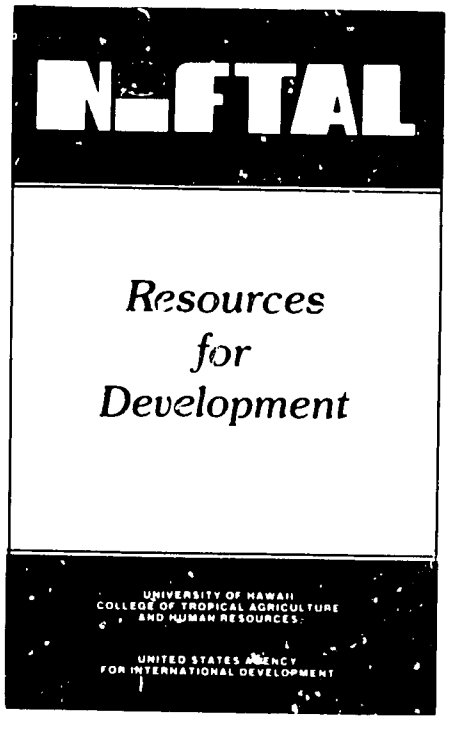
GENERAL BACKGROUND



Now in its tenth year, the NifTAL Project was established in 1975 at the University of Hawaii under contract with USAID. Though major funding has been through grants from USAID, additional funding has been provided from NSF, NAS, Unesco, USDA, the Science Advisor's Office-Aid, the Donner Foundation, and the Rockefeller Foundation as individual grants to NifTAL researchers.

PROGRAM

NifTAL's overall goal is aiding LDC farmers to reduce their dependence on nitrogen fertilizers needed to produce increased quantities of high protein food. This is accomplished by research and by assisting Rhizobium and legume programs throughout the tropics to more effectively exploit the Rhizobium-legume symbiosis.

Achievement of the overall project goal required that NifTAL develop:

1. A comprehensive collection of Rhizobium germplasm containing efficient strains adapted to tropical conditions which could be distributed to researchers and inoculant producers in the tropics.

2. Improved techniques and systems for ensuring dependable effective nodulation and maximum utilization of legume-fixed nitrogen in cropping systems.

3. A body of LDC researchers and inoculant producers trained in BNF technologies.

The development of these resources comprises the specific objectives of the NifTAL Project. Meeting these objectives required an integrated approach involving research, outreach, and training activities.

Ten categories of activities (objectives) were undertaken: rhizobial germplasm development, rhizobial antisera services, international network trials, legume inoculant development, management of biologically fixed nitrogen in cropping systems, soil stress tolerance research, economic assessment of BNF technologies, regionalization of BNF support, training and communication, and country and inter-agency support.

ORGANIZATION

The worldwide NifTAL Project is staffed by a multinational team of microbiologists, agronomists, soil

scientists, training and communication specialists, research associates, and support staff. NifTAL maintains permanent stations and staff in Maui; Other staff are outposted to Bangkok, Thailand; Mount Makulu, Zambia, and expense support is provided to an INLIT coordinator in India.

NifTAL-Maui is staffed by nine researchers, ten research associates, and fifteen support staff organized into four sections: 1) The Research Section conducts research in agricultural BNF technology at the NifTAL headquarters Hamakuapoko, and at three field stations on Maui; 2) The Training and Communication Section offers graduate assistantships for PhD candidates, mounts specialized in-country training courses for researchers, extension leaders, and production personnel, hosts visiting scientists and intern trainees, and also publishes the BNF BULLETIN, a continuing bibliography, training aids, and technical papers; 3) The International Outreach Section coordinates the extensive International Network of Legume Inoculation Trials, and offers in-country BNF program development support to national governments and aid agencies. In addition, the BNF Resource Center at Bangkok, Thailand, in cooperation with the Thailand Department of Agriculture, offers all NifTAL services in the South and S.E. Asia region, and conducts regionally focused research and training; and 4) The Administrative Section manages operations on Maui and coordinates worldwide activities.

SUMMARY OF ACCOMPLISHMENTS

1984 was an interesting and challenging year for the NifTAL Project. New training courses were offered and linkages established. And, a wide variety of laboratory, greenhouse, and field experiments were run. These activities were directly related to our project goals of doing research and assisting legume programs throughout the tropics to more effectively exploit the legume-Rhizobium symbiosis.

The BNF Resource Center in Bangkok surged into action as an office and BNF library were established, a laboratory set up and laboratory technical staff trained, linkages developed with several international centers and development organizations, support requests answered, and an Azolla Workshop given at the BNFRC in January.

NifTAL offered several other courses during the year including two new courses: a BNF research/extension training course, held in cooperation with BARC and BARI in Bangladesh for 35 participants, and a 3-week legume inoculant production course held at Maui Headquarters for inoculant producers from ten countries.

Other co-sponsored courses were a 2-week N-Fixing Tree Association Workshop for 12 participants from the Pacific Islands and Mexico and an Inoculant Production Course held in Uruguay for 30 researchers.

Two visiting scientists--one from Ghana and one from Peru--served extended internships at NifTAL. Sixteen other researchers interned for short periods during the year. Graduate students from 6 countries worked toward advanced degrees and one Nepalese student attained his PhD.

Several new publications, along with audio-visual productions and posters, were released in 1984. The document collection expanded, now numbering nearly 8,000 documents. Over 1,000 requests for documents and catalogues were answered. And the BNF BULLETIN mailing list grew with 1,900 readers in 100 countries now receiving 3 issues annually.

Linkages between NifTAL and international development agencies worldwide continue to be formed through cooperative agreements, assistance as instructors in courses, and through joint experiments. NifTAL staff members have answered many requests for consultancies from such agencies as USAID, NAS, FAO, and CIAT.

As part of a subcontract on the ZAMARE Project, a NifTAL inoculant technician spent several months in Zambia supervising and assisting in the production of 2,200 bags of inoculant for inoculating 5,000 hectares of soybean.

Completed results returned from International Network of Legume Inoculant Trials increased by 30% over returns received in 1983 bringing the total number of completed "A" trials to 200. Compilation of all INLIT data was completed in mid year and results disseminated in a Bulletin which was sent to all cooperators. "A" tests were requested and sent out to 141 cooperators representing all INLIT regions. Hundreds of bags of inoculant and thousands of bags of sterile peat were requested and distributed.

An evaluation of network procedures was conducted through questionnaires sent to cooperators. The evaluation helped identify specific problems cooperators had experienced.

Highlights of the inoculant development program were: production of a legume inoculant production manual for the course held in December at NifTAL; a pocket manual, produced cooperatively with FAO, on inoculant use for LDC extension workers; and completion of the pilot scale inoculant production plant at NifTAL's Haleakala site. Experiments conducted this year looked at alternate carriers and validation of a new inoculant production technique.

The Rhizobium Germplasm Resource Catalogue was updated. Over 40% of the 1774-strain collection has been lyophilized. This year, nearly 600 cultures were sent in response to 73 requests from 34 countries.

Experiments were conducted to: characterize native rhizobial populations in terms of population numbers and effectiveness; explore how the host controls and allocates nodule sites to particular rhizobial strains; and characterize selected strains through competition and cross-inoculation studies.

Made available this year was a catalogue listing antisera stocked in the NifTAL serum bank. This catalogue will give researchers using antisera to identify strains of Rhizobium better access to the collection. Antisera were produced to replenish stocks and storage tests compared the differences between antisera that were lyophilized or those frozen in 50% glycerol.

Our goal is to reduce dependence of small farmers on costly nitrogen fertilizers for production of high quality food. This is accomplished by doing research and providing assistance to Rhizobium and legume programs throughout the tropics.

Responding to recommendations of the Rodale Workshop, experiments were conducted to quantify N_2-fixation in rotational and intercropping systems. Additional studies examined N transfer from a legume to a non-legume via VAM fungi using a split root technique and compared methods for measuring N_2-fixation by trees and crops.

Some significant findings include: VAM fungi mediated N uptake by corn grown with soybeans; ^{15}N-depleted material was successfully used to quantify N_2-fixation by Leucaena Leucocephala, Albizia lebbek, cowpeas, and beans; % N in beans derived from fixation varied from 24 to 48% depending on whether corn or uninoculated soybeans were used as reference plants for ^{15}N calculations; cowpeas fixed 10 kg N/ha or 55% of total N harvested with the % N from fixation increasing with planting density; and inoculation of cowpeas, beans, and soybeans with Azotobacter or azospirillum and/or rhizobia did not enhance growth over inoculation with rhizobia alone.

NifTAL subcontracts at UC-Davis and University of Wisconsin produced several publications. At UC-Davis, soil stress experiments screened Sesbania and Leucaena for NaCl tolerance.

A research associate from the University of Wisconsin has been outposted to Khon Kaen University, Thailand, where he will be testing a farm model designed to measure the economic benefits of BNF to the small LDC farmer.

UNIVERSITY OF MINNESOTA

DAVIS, CALIFORNIA

ACKNOWLEDGEMENTS

The University of Hawaii acknowledges the continuing support by the USAID for NifTAL, initially through Contract AID/ta-C-1207 (1975-1982) and currently under DAN 0613-C-00-2064-00. NifTAL has grown in size and capability over the years. The USAID core budget for the NifTAL Project was the catalyst permitting establishment of an integrated, multi-disciplinary program now augmented by many other awards from public, private and international sources.

The University also recognizes the strong support of the Mayor of Maui and the Maui County Council for provision of the 25-acre NifTAL research campus.

BNF RESOURCE CENTER

Objective

- The BNFRC focuses on providing a strong foundation for research, training, technical aid, and information dissemination. Because it is a project oriented to the needs of a specific region, strong links are established with other institutions located in South and Southeast Asia which are also engaged in BNF research and development projects.

1984 was a busy first year which saw the development of many exciting programs. The following is a list of some of those accomplishments.

The establishment of a functional office and BNF library capable of international communication and information dissemination. All information is accessible through microcomputer database management.

Training was provided for a technical staff oriented towards general rhizobiology and analytical procedures. This staff will form a service section (supply of Rhizobium strains and antisera, inoculants, special analysis, etc., to requesting scientists) and provide assistance in research projects to be conducted in collaboration with regional scientists. Set up a laboratory capable of production and dissemiation of bacterial cultures and antisera.

Application of BNFRC funds implemented the use of facilities and equipment sitting idle--because of lack of funds--in the legume inoculation production plant within the Soil Microbiology Branch. Functionalizing of the plant resulted in additional funds from the Thai Department of Agriculture being made available to complete the setup of the plant.

Introduced was the concept of the BNF Resource Center and the nature of its activities to the region. This was accomplished by visiting institutions in Pakistan, India, Bangladesh, Nepal, Indonesia, Philippines, Burma, Singapore, Australia, and throughout Thailand. Strong linkages were developed with other international centers and development organizations including ACIAR, AVRDC, FAO, IADS, IDRC, ICRISAT, and IRRI. A strong network with these institutions will be essential in establishing BNF information exchanges and coordination.

A workshop on the use of Azolla in tropical cropping systems was presented at the Resource Center in January. Twenty-five participants from six regional countries attended the workshop which was coordinated by Dr. T. Lumpkin. An upgraded version of another course, the first course designed and conducted to inform extension workers of the benefits and limitations of the use of BNF in cropping systems (held in Bangladesh), is slated to be taught in the region. Planning was begun to develop extension courses to be held in Thailand and the Philippines in 1985.

Assisted in the development of facilities to house and train interns at the Rhizobium Building. Selected and assisted in the placement of the recipient for the NifTAL Ph.D. fellowship (N.A. Mirza, Pakistan).

Published the first issue of the Regional BNF newsletter which is scheduled to be mailed three times yearly to over 200 scientists in the region.

Training course participants observe a strain selection technique.

Services

Assist regional national BNF programs; provide short-term, intern, and graduate degree programs for LDC scientists to work with various aspects of BNF and its application in cropping systems; design and implement relevant legume inoculant production and distribution systems; promote local scientist's participation in legume inoculation trials (INLIT); provide selected rhizobial cultures, inoculants, and Azolla strains adapted to regional constraints to area scientists; link regional scientists through workshops, information/document exchange, and the BNF regional newsletter; and provide technical assistance in the optimal use of BNF in farming systems.

TRAINING AND COMMUNICATION

Objective

- to disseminate NifTAL research output and transfer professional skills and technical information for research, extension and production enterprises required to assess and use technologies based on biological nitrogen fixation for crop production in the tropics.

Accomplishments

Information Dissemination

NifTAL research output is disseminated through journal publications and conference/workshop presentations. See Appendices C (Bibliography 1976 - 1984) and D (Abstracts - 1984).

During 1984 there were 327 requests for documents authored by NifTAL staff, and 250 copies of the Rhizobium Strain Catalogue were distributed.

Past and current technical information on legume nitrogen fixation is available to researchers in developing countries through the NifTAL Document Collection. This collection now has 7,800 entries, of which nearly 800 were added in 1984. The periodic bibliography distributed with the BNF BULLETIN continues informing researchers of articles and documents available free of charge to developing country BNF workers. In 1984, NifTAL serviced 483 requests for documents from researchers in 54 countries.

NifTAL's BNF BULLETIN mailing list continues to grow with 1,900 readers in 100 countries receiving 3 issues of the BULLETIN annually. The newsletter sustains awareness by an international development audience of projects in progress worldwide that impact on the use of BNF in agriculture. The BULLETIN is also a vehicle for exchange of ideas between researchers and decision makers.

Over 200 scientific and citizen visitors came to NifTAL this year. A visitor list is included in Appendix F.

Instructor and participants in the inoculant production course.

Courses and Workshops

Commercial Scale Legume Inoculant Production Training Course - A three-week legume inoculant production course was held at the Maui headquarters. A proto-type of future courses, this first time offering attracted 10 participants from 10 countries. The schedule of exercises and lectures in shown in Appendix G.

Azolla Course in Thailand - Approximately 25 participants from several South and South Asian countries attended this course on Azolla technology. Dr. T. Lumpkin coordinated the course.

Bangladesh Extension Course - NifTAL cooperated with the Bangladesh Agricultural Research Center (BARC) to mount the first BNF research/extension training course. In addition to developing a training format for similar training programs, the objectives of the course were to create awareness of BNF's potential; upgrade research and extension worker capabilities; and create linkages between the groups. The course's outline is shown in Appendix G. Nearly 35 trainees attended the course. Senior scientists participated from BARC, BARI, North Carolina State University, and NifTAL. As a result of the Course, a manual was co-produced by BARC, IADS, and NifTAL. (See Appendix G.)

Production and Management of Nitrogen-Fixing Trees - NifTAL assisted the Nitrogen Fixing Tree Association (NFTA) in organizing the course which covered all areas of tree research and production. Twelve participants from the Pacific Islands, the Philippines, and Mexico participated in the two-week course which included a field trip to NifTAL Headquarters on Maui. Seven groups co-sponsored this workshop.

Latin American Inoculant Production Course - NifTAL co-sponsored a course on the "Development, Production, and Use of Inoculants" run by the Secretary of Agriculture, Plan Agropecurio of Uruguay, from 20-31 August 1984. Thirty researchers and inoculant producers from eleven Latin American countries attended. NifTAL provided support for participants and the INLIT Latin American coordinator presented several lectures. The favorable response of attendees will undoubtedly lead to the course being offered again, most likely every other year.

Caribbean BNF Symposium - NifTAL co-sponsored the First Regional Symposium on BNF for the Caribbean held by the Secretary of Agriculture of the Dominican Republic from 1-7 August.

African Training Course - A host institute was selected and communication established to finalize arrangements for the 6-week legume/Rhizobium Training course to be held in Africa in 1985. However, budget constraints in 1985 and 1986 make it improbable that such a course will be undertaken. NifTAL is currently seeking support for such a course.

Serology Course in China - During 1984, a serology course has been under discussion. This course would be a cooperative effort of the Chinese Academy of Science, FAO, and the NifTAL-MIRCEN. Discussions are expected to continue into 1985.

Frank Sorwli

Interns

Serving extended internships at NifTAL during 1984 were senior scientists from Ghana and Peru.

Tomas Tello Reyes

Tomas Tello Reyes (Chiclayo, Peru), a forage crop specialist, worked on assessing the effects of rhizobial and mycorrhizal inoculation on the growth of three N-fixing trees - Prosopis pallida, Leucaena leucocephala, and Gliricidia sepium. Another aspect of his work was comparing forage legumes under different P levels.

Frank Sorwli (Accra, Ghana), lecturer in the Crop Science Department, studied the underexploited legume, Bambara groundnut (Voandzeia subterranea). He worked with NifTAL microbiologist P. Somasegaran. They investigated the variation in effectiveness of native rhizobia isolated from the nodules of Voandzeia plants grown in Kenya and Hawaii. Rhizobium affinites of Voandzeia were also studied using rhizobia isolated from other legume species. He also studied host-strain interaction in the Leucaena-Rhizobium symbiosis using three Leucaena species. A multi-strain Rhizobium inoculant was used to inoculate the three species and strain competitions were studied.

Others interning for short periods of time were from Burma (4), Sri Lanka, Bangladesh (4), India (3), Thailand (4), and China. See participant list, Appendix E.

Graduate students working on advanced degrees at the University of Hawaii are from USA, Zambia, Liberia, India, Bangladesh, and Sri Lanka. One Nepalese student attained his Ph.D. in Agronomy and Soil Science from the University of Hawaii in mid-1984. See participant list Appendix E.

Publications

Inoculant Production Manual - In conjunction with the Legume Inoculant Production Course, a manual was developed. Written by J. Burton, the 128-page document covers all aspects of inoculant production. The Manual's table of contents is shown in Appendix H.

NifTAL/FAO BNF Pocketbook - This pocketbook was submitted to FAO and scheduled for publication in March 1984. FAO/Rome has confirmed that publication has not taken place, but completion is expected in early 1985.

The NifTAL Training Manual - The training manual is available in a draft form and negotiations are underway to select a publisher.

Audio-Visual Productions - New "What is NifTAL" Slide-Tape Program - This version of What is NifTAL is directed toward the scientifically oriented visitor. Slides sets were developed and sent to developing country researchers for their use. Two posters were prepared for poster sessions at major conferences.

INTERNATIONAL OUTREACH

Objective

- to apply the physical and human resources established at NifTAL on USAID central funding to specific projects requested by USAID missions of individual countries, or by other development agencies. Such projects are integrated into NifTAL's program, but are separately funded.

Accomplishments

Carribean BNF Symposium

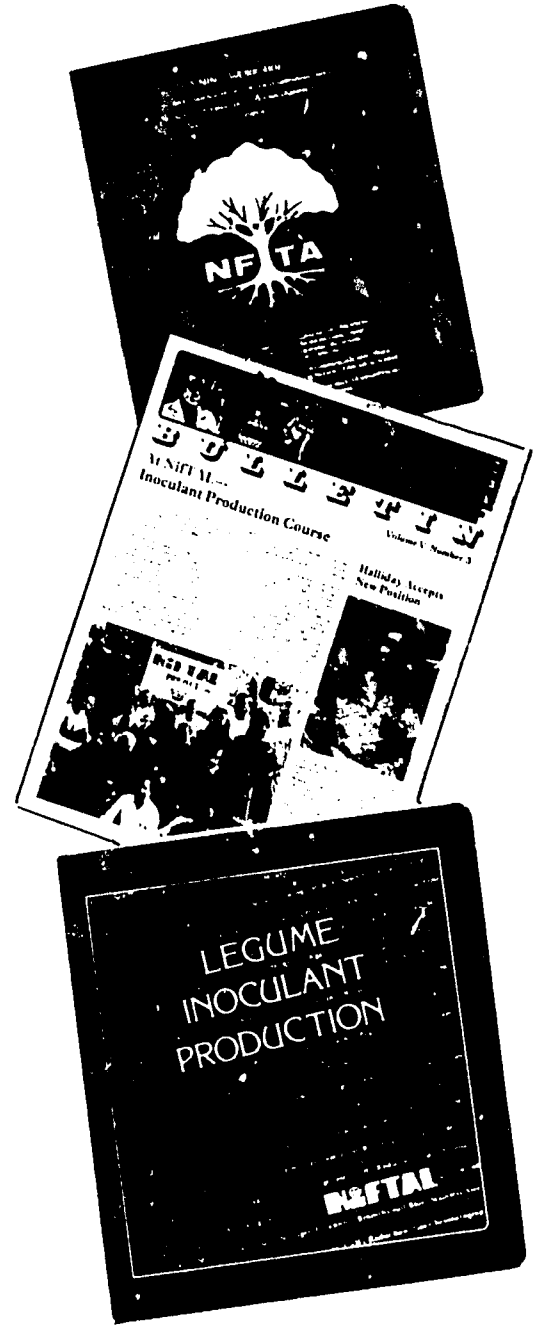
NifTAL co-sponsored the First Regional Symposium on Biological Nitrogen Fixation for the Caribbean held by the Secretary of Agriculture of the Dominican Republic from 1-7 August, and the XII Latin American Rhizobium Conference held from 20-26 October in Campinas, Brazil.

Cooperative Effort in Latin America

The Latin American coordinator assisted, along with CIAT, the Boyce Thompson Institute, the International Fertilizer Development Corporation, the UNDP and the MIRCEN, Porto Alegre, in a meeting to design a research/training project aimed at evaluating a new inoculant. NifTAL was invited to attend by CIAT and the UNDP. As a result of the meeting, NifTAL will participate in the project by training two teams of BNF researchers from the Carribean. In addition, the NifTAL training manual will be used in the training portion of the program. Funding for NifTAL's participation will come from CIAT.

ALAR Participation

Due to our extensive networking experience, NifTAL was invited by the Latin American Rhizobium Association (ALAR) to participate in a planning session to develop a Network of BNF biotechnology centers in Latin America. Alar representatives from 15 nations attended the 3-day meeting and drafted a



proposal, which was submitted to the UNDP Biotechnology Program, to establish the network.

Inoculant Quality Tested in Egypt

As part of the service activities of the international outreach section, NifTAL performed quality control tests on the peat based soybean inoculant produced in Egypt for 1984. The inoculum proved to be of good quality (>10^7 cells/g peat) and produced yields were up to 200 kg N ha^{-1} could be fixed through inoculation technology.

Zamare Project

NifTAL has a subcontract from the University of Illinois to assist in the development and improvement of an inoculum industry in Zambia. While the early focus of the project was concerned with testing and production of soybean inoculants, the facility will be able to provide inoculants for a wide variety of legumes currently in use or exotic species with potential in the region. In addition to placing a permanent senior microbiologist at the Mt. Makulu research station, NifTAL offered technical assistance to Zambia during the peak production season by posting an inoculant production assistant to the Mt. Makulu facility. NifTAL-Hawaii serves the Zambia facility in a variety of logistical and technical capacities. During 1984 a major renovation of the inoculum lab was completed by the Illinois team according to special design requirements requested by NifTAL personnel.

ining peat in Kataba Valley

For the first time, almost 30% of needed inoculum was produced with peat mined in Zambia's Kataba Valley. The peat proved to be equal in quality to standard inoculum peat used in the U.S. Studies are continuing on the long-term survival of rhizobia in Zambian peat. If these test results are positive, it may be possible for Zambia to develop an export inoculum industry that serves the needs of the region.

A field experiment was conducted in Zambia to examine strains of Rhizobium japonicum on soybean cultivars in use or being developed for Zambia. Results indicated that large responses to inoculation (in excess of 1,500 kg/ha above controls) are possible. Increased yields from inoculation were observed on both cultivars of North American origin and those bred to nodulate with indigenous rhizobia and therefore not requiring inoculation.

INTERNATIONAL NETWORK TRIALS

Objective

- to determine, under realistic field conditions, whether the yields of tropical legumes can be increased by inoculating them with selected strains of Rhizobium.

Accomplishments

Results from 95 "A" trials were returned and analyzed as compared to 65 "A" trials in 1983. The total number of completed trials is approximately 200 (see Appendix A, Table 1).

A compilation of all INLIT data collected to date was made during the summer of 1984 with the assistance of Dr. Foster Cady. A summary of the results of the "A" tests was presented in the Cooperators Bulletin #3 (Appendix A, Table 2).

Networks

Asian

Data from an additional 81 trials were returned to NifTAL during 1984. There were 106 requests for "A" tests and 1,760 bags of inoculant sent to 14 Asian countries.

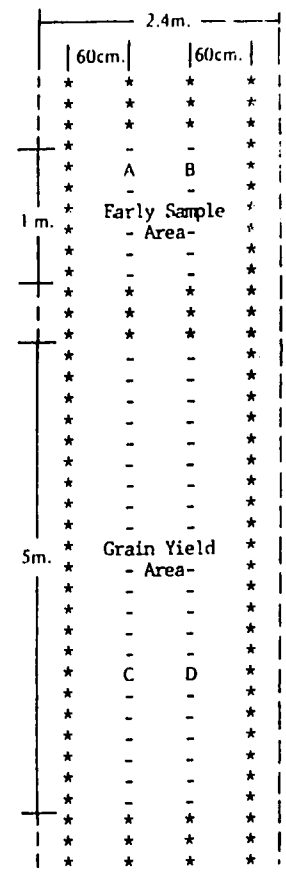
The Asian coordinator responded to a request from the Pakistan AID Mission to consult on the organization of a country-wide BNF program. He also responded to a request to participate in organizing and offering a BNF extension course in Bangladesh. This course was the prototype for one which will be offered again in Thailand in 1985. At the invitation of the Burmese Ministry of Agriculture, he offered a consultancy on implementation of a countrywide BNF program.

African

A total of 88 bags of inoculant and various laboratory supplies were delivered to nine African countries. Special requests for a total of 1,000 bags of sterile peat were sent to three countries. Data from 1 "A" test was returned. Six "A" tests were mailed out.

The network coordinator for Africa assisted various cooperators and expanded our outreach during his visits to Africa. In Kenya, he consulted with S. O. Keya and reviewed the inoculant facility at University of Nairobi (Kabete Campus).

Pulses



A & B = early sample
C & D = yield determination

The early sample (except species) is taken when 50% of the plants are flowering. Ten plants are harvested from A and B. At maturity, seed is harvested from C and D. For other row spacings adjust accordingly.

Cooperative experiments involving N_2 fixation and cropping systems were arranged with individuals in Ghana and at the University of Khartoum at Shambat. The purpose of these experiments is to assess inoculation responses and determine the potential amount of N_2 fixed by legumes in local cropping systems. NifTAL's role will consist of technical support in experimental design, analysis of samples, and publication of results.

Latin American

Network cooperation greatly expanded in 1984 with 254 bags of inoculant sent to 16 different countries. Twenty-six "A" tests were mailed out to various cooperators and 11 completed tests were returned for analysis.

The Network coordinator met with the Latin American INLIT collaborators in Brazil in October 1984 during the 12th RELAR. The meeting involved not only active participants in the INLIT program but interested researchers as well. Test data for several "A" trials was presented by the cooperators in the INLIT Program. The general response of the cooperators to the INLIT program was very positive, however, they requested special assistance in serology and better communication between NifTAL and the cooperators.

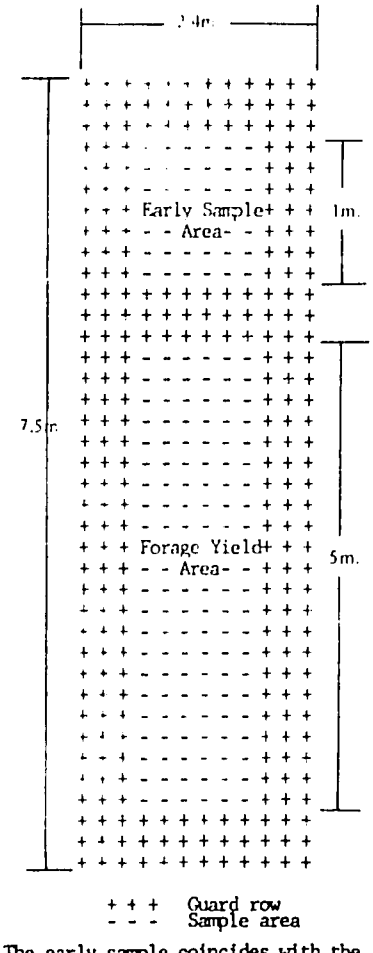
Middle-Eastern

Nine "A" tests were requested and delivered; two tests were returned for analysis. A total of 30 bags of inoculant were mailed. The Network coordinator visited the Egyptian Major Cereal Project and provided technical assistance to their inoculant quality control program. He assisted Mr. haples of Care-Sudan Leucaena Project by providing .noculant. At the University of Sudan, he worked with Dr. Hago El-Magid on a manuscript--the result of collaborative research begun while Dr. Magid was an Intern at NifTAL, set up four INLIT "A" experiments, and discussed NifTAL participating in graduate student research projects. In Saudi Arabia, he reviewed and made recommendations to a soybean project.

Services

Over 44 countries requested 1,520 bags of inoculant and 1,600 sterile bags of peat. Shipments included not only inoculant but seed, cultures, and sometimes laboratory supplies for use in quality control of inoculant and its production. Inoculants sent and their destination countries are listed in Appendix A, Table 3. In Appendix A, Table 4, inoculants sent are listed by species.

Forages



The early sample coincides with the first cut. The area sampled can be modified to suit the cutting method. Border should be 50cm minimum. After sampling, the entire plot should be cut to a uniform height.

Questionnaires, included with the Cooperators Bulletin, were sent out in September to 200 cooperators worldwide; 20% were returned. The questionnaire allowed evaluation of network procedures and assisted in determining any specific problems the cooperators may have had with the tests.

INOCULANT DEVELOPMENT

Objective

- to develop systems for improved inoculant delivery, and for ensuring dependable, effective inoculation in the field.

Accomplishments

Research

Suitable peat is unavailable in many developing countries, therefore a need exists for alternate carriers. In September, 1984, a soybean experiment was conducted to compare the efficacy of granular inoculants made from coal and charcoal to liquid inoculant, regular granular peat inoculant, regular powdered peat coated on silica sand, and seed inoculation with regular powdered peat inoculant with gum arabic as sticker. There were no significant differences between treatments in the shoot dry weights at 50% flowering. However, significant differences ($p < 0.001$) were observed in nitrogenase activity and nodule dry weight. Nodule dry weight and nitrogenase activity were highly correlated ($R^2 = 0.99$; $p < 0.001$). Results showed that granular inoculants made from coal and charcoal are as effective as granular inoculants made from peat and that silica sand coated with powdered peat inoculant can be used as a granular inoculant. Nodules from this inoculation experiment are being typed to determine competition amongst the three R. japonicum strains used in the inoculant.

Course instructor discusses Rhizobium storage methods.

Previously (NifTAL Annual Report 1983), the validation of a new inoculant production technique (a dilution procedure) which eliminates the need for large fermenters was reported. Because the design of a practical system was lacking, we now have designed a prototype that can produce large amounts of sterile water for dilution of liquid cultures. Sterilization is achieved by pumping water through a 0.2 micron bacteriological filter. It is then stored in 25-liter presterilized carbuoys in which cultures are diluted prior to injection into presterilized peat.

Participants prepare inoculant fermentors.

In 1984, this prototype production system was field-tested in Zambia as part of the Zamare Project. Using this system, NifTAL produced 2,200 bags (400 g per bag) of high quality inoculants (1 - $5x10^9$ rhizobia per g inoculant) for approximately 8,800 hectares of soybean.

NifTAL's Haleakala Inoculant Production Facility for development of low cost production systems, carrier testing, and training LDC inoculant producers was completed and a three-week course on inoculant production was held there from 3-21 December 1984. Low cost fermenters of varying capacity (8, 16, and 37 gallons) were used in this training course. The Haleakala facility is now complete with a hammer-mill, autoclave, paddle-mixer, and a microbiological work lab.

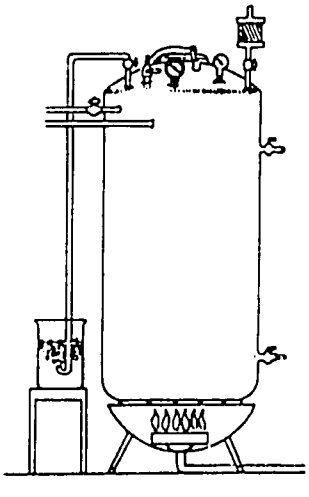
Publications of the following are being prepared.

a) A methods paper on the small lab-fermentor,

b) A methods paper on the production of research inoculants at NifTAL for publication as a technical bulletin, and

c) A technical not on the single transer chamber.

Because of NifTAL's concern that developing country farmers be able to obtain high quality inoculants, the Inoculant Quality Incentive Program (IQIP) was instituted. The program called for a Board of Standards, definition of uniform standards for inoculant products, award of "seals of approval" to be carried only by products of companies subscribing to the Board's standards, and creation of an awareness among users and retailers that the seal is a sign of quality they can trust. A program draft has been written and critiqued by several Rhizobium scientists.



A list of inoculant producers has been compiled and is being expanded as data is gathered. The program has been publicized and interest in the IQIP has been expressed by more than a dozen inoculant producers worldwide.

A computer conference to discuss implementation of the program was initiated and received strong attention and support by interested outside scientists and institutes. The conference was only partly successful, however, in large part because of the technical difficulties of getting participants on-line. Unfortunately, no alternative forum for discussion of the program was proposed because of lack of funds for this purpose.

RHIZOBIAL GERMPLASM

Objective

- to maintain a Rhizobium germplasm resource comprised of authenticated, characterized rhizobial strains from a wide array of plant hosts and diverse geographic origins, from which to select strains which nodulate agriculturally important legumes under conditions prevailing in the tropics.

Accomplishments

Research

Native populations of Rhizobium consist of a wide variety of strains for a given legume species. These populations may vary in both the number of invasive rhizobia and their effectiveness for N_2-fixation with a particular legume. As part of the development of the ecological model for explaining inoculation responses, a two-year project was undertaken to characterize native rhizobial populations in terms of population numbers and effectiveness.

Most probable numbers (MPN)[1] of invasive Rhizobium in four Hawaiian soils

Soil Series	[illegible]	A. hypogaea	[illegible]	[illegible] unguiculata	[illegible] vulgaris	L. leucocephala
	----- [illegible] Rhizobium [illegible] soil)$^{-1}$ -----					
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		
[illegible]	[illegible]	[illegible]	----	[illegible]	[illegible]	
[illegible]	[illegible]	----	[illegible]	[illegible]		[illegible]

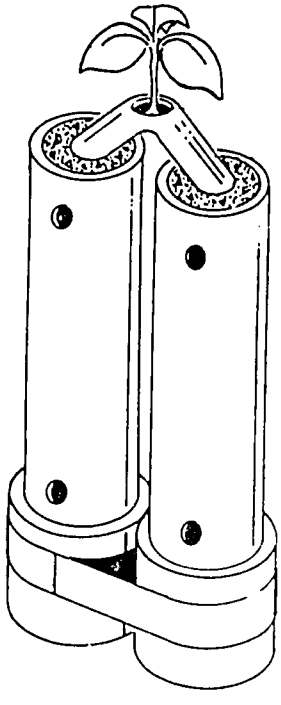
[1] [illegible] that inoculation significantly increased [illegible] of [illegible] respectively.

[2] [illegible] Rhizobia [illegible] soil [illegible].

A series of inoculation trials were conducted with six legume species in four different Hawaiian soils. Inoculation resulted in increased N_2-fixation only when either the soil population was completely ineffective or the numbers of effective native rhizobia were less than 30 invasive cells per gram soil. The magnitude of the inoculation response increased dramatically when soil numbers were below one rhizobia per gram of soil. Tests for effectiveness of random nodule isolates of uninoculated treatments revealed that most rhizobial populations consisted of strains with a range of effectiveness for N_2-fixation. Most populations had some strains that were superior to the selected inoculum strains. However, even when the inoculum strain was significantly more effective than the average, soil strain inoculation responses were not observed when soil populations were high.

So that future technology may improve the establishment of inoculum strains in the field, it is imperative that we understand how the host controls and allocates nodule sites to particular strains. To do this, two experiments were conducted using a split-root technique and radioactive tracers to determine how quickly after inoculation the host

(soybeans) selectively partitions carbon to the site of inoculation. Different inoculation treatments were applied to half the root system and shoots were labeled with C^{14}-CO_2 at intervals after inoculation. The data from these experiments are currently being processed.



A split root growth system

Characterization of selected strains listed in the 1984 Rhizobium Catalogue was continued. Four species of Leucaena (L. lanceolata, L. pulverulenta, L. diversifolia and L. shannoni) were tested for cross-inoculation membership. A three-strain mixed inoculant for these species was developed. A competition study using the mixed inoculants was run and the nodules are presently being typed. Similar cross-inoculation studies were done on Gliricidia sepium which has been targeted as an important tree species by development agencies. A three-strain mixed inoculant is now available for Gliricidia and awaits further field testing. Multi-strain (3) inoculants for the moth bean (Vigna acontifolia), hyacinth bean (Lablab purpureus), rice bean (Vigna umbellata) and the yam bean (Pachyrhizus erosus) were tested in the greenhouse.

Services

The Rhizobium Catalogue was further revised and updated. All strain information is now computerized and regular updating on strains is being continued. Approximately 41% of the collection (total 1/74) is now lyophilized and lyophilization of the remaining germplasm will continue.

In 1984, NifTAL sent out 583 cultures in response to 73 requests from 34 different countries. (See Appendix A, Table 5.) This amounted to a 14% increase in requests over 1983. More than 82% of the requests came from scientists in developing countries.

RHIZOBIAL ANTISERA

Objective

- To establish a serum bank housing antisera for identifying the strains of Rhizobium recommended for the economically important legumes under test in the International Network of Legume Inoculation Trials (INLIT).

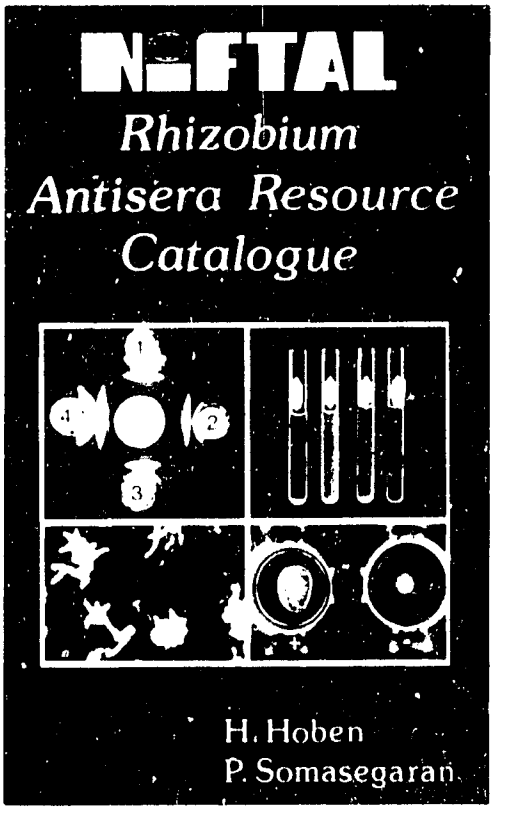
Accomplishments

Research

Approximately 1.2 liters of Rhizobium antisera and 1 liter of goat-anti-rabbit antisera were produced to replenish antibody stocks and to increase the volume of the serum bank. Antisera were produced for 3 strains of Phaseolus lunatus which is a legume recently added to the INLIT list and for three strains of Rhizobium meliloti, two of which will replace INLIT strains which are not antigenically distinct. Four Rhizobium antisera were conjugated with FITC to replenish antisera stocks for the direct fluorescent antibody (FA) technique and FITC-conjugated goat-anti-rabbit antisera were prepared for use in the indirect FA technique.

The serological reactions of all cultured cells of the INLIT collection have been compared with their corresponding bacteroid reactions using the indirect FA technique. All results were positive.

In an antiserum storage test, four FITC-conjugated antisera were separated into two batches. Batch 1 was lyophilized and stored in sealed glass ampoules at room temperature, Batch 2, in a liquid form, was stored as a 50% mixture with glycerol at -20 degrees C. After 7 months, Batch 1 dropped in FA titre, while the titre of Batch 2 remained the same.



Services

A catalogue has been prepared and is available which lists the antisera stocked in the NifTAL serum bank. A yearly update is anticipated. A compact manual describing production and use of antisera is still in preparation.

Requests for antisera have incresed over last year. A total of 394 ml were supplied to researchers in several developing countries (see Appendix A, Table 6).

N MANAGEMENT IN CROPPING SYSTEMS

Objective

- to derive an improved understanding of how to manage tropical cropping systems to optimize the benefits from nitrogen fixed biologically.

Accomplishments

Research

BNF scientists at a NifTAL field site.

* Compare field methods for quantifying nitrogen fixation in tropical soils when yield potential is varied by phosphorus application.

A field experiment was conducted, in a highly weathered, P-fixing Ultisol, to evaluate methods of quantifying N_2-fixation by Phaseolus vulgaris under different yield potentials. Phaseolus, both inoculated and uninoculated, and corn were grown at four levels of P in a split-plot design. ^{15}N was applied to subplots. By employing this design, N_2 fixation can be measured three ways: 1) the difference between total N in inoculated and uninoculated bean; 2) isotope dilution using corn as a non-fixing reference crop; 3) isotope dilution using uninoculated bean as a reference crop.

There was a four-fold increase in dry matter and nitrogen of corn and beans with the addition of phosphorus. Nitrogen accumulation followed a similar trend in the non-fixing controls. A soil N mineralization study is in progress to determine whether increased N uptake with P fertilization was due to increased exploration of the soil profile by the plant or an increase in the rate of N mineralization by soil microorganisms. Isotope analysis remains to be completed on plant tissues so that the different methods of measuring N_2-fixation may be compared.

* Compare the response to inoculation of four legumes grown in the field and measure the amount of N_2 fixed and the residual N benefits to the following non-legumes.

A field inoculation trial was conducted at the Kuiaha field site with Arachis hypogaea, Glycine max, Phaseolus vulgaris, and Vigna unguiculata. One purpose of this work was to assess how native soil rhizobia affected the inoculation response of different legume species. Another purpose was to measure the amount of N_2 fixed by species and determine what residual N benefits would be available to subsequent non-legume crops. Nitrogen fixation was determined by the isotope dilution method using uninoculated soybean and kidney bean and corn as non-fixing reference plants. In the inoculation trial, soybean and, to a lesser degree, kidney bean responded to inoculation. There were no Rhizobium for these species in the native population. Cowpea and peanut did not respond to inoculation because of competition from native rhizobia. There were $1 \times 10^2/g$ rhizobia in the soil.

Substantial N from mineralization during crop growth probably reduced the extent of inoculation response in kidney bean which had a lower yield than soybean. Isotope analysis remains to be completed. In 1985, the experimental plots will be planted with corn to measure the effects that species and inoculation have upon residual N. Three plots which remained fallow in the first experiment will be used to equate the residual N benefit with applied urea.

* Improve the establishment and N_2-fixing ability of important tree legumes on phosphate-fixing soils through the use of vesicular-arbuscular mycorrhizal (VAM) fungi.

Studies have been completed on the influence of two tropical isolates of VAM fungi on nodulation, growth and nutrient uptake of Sesbania grandiflora. The results of this investigation have clearly established that VAM fungi vary in their sensitivity to toxic ingredients resulting from soil sterilization and that comparison of strains and species of VAM fungi under this condition may not be valid. The results have also demonstrated that nodulation, growth and nutrient uptake of Sesbania was significantly improved by inoculation of non-sterile soil with either Glomus fasciculatum or G. mosseae. These findings are the subject of a manuscript which is being submitted for publication in Applied and Environmental Microbiology.

Experiments involving the influence of G. fasciculatum on Leucaena leucocephala have yielded results that were different from those observed for Sesbania. Inoculation of non-sterile soil with G. fasciculatum did not lead to improved nodulation, growth or nutrient uptake of Leucaena.

Experiments were also completed that were designed to determine the best support medium for multiplying mycorrhizal cultures, and for growing tree legume seedlings maximally infected with VAM fungi. A 2:1 (wt/wt) mixture of mansand and Wahiaha soil is found to be ideal for these purposes.

In experiments designed to determine the level and duration of exposure necessary to inactivate VAM activity in crude inocula for use in control treatments, it was found that up to 100 g of cycloheximide applied to crude inoculum of G. fasciculatum for up to 48 hours was not effective in eliminating VAM activity.

An example of the effect of nutrients on plant growth.

A preliminary experiment designed to test the feasibility of using VAM fungi for establishing cowpea on an Oxisol subjected to simulated erosion has just been completed and the data is being analyzed.

* Evaluate the use of ^{15}N depleted material for assessing nitrogen fixation by Leucaena leucocephala and Albizia lebbeck

Intercropped legumes and corn—a typical growth system in developing countries.

Leucaena leucocephala (haole koa) and Albizia lebbeck (woman's tongue) were grown in Leonard jars supplied with different levels of ^{15}N-depleted ammonium sulphate and inoculated with appropriate Rhizobium strains. The % N derived from ammonium sulphate and from N_2-fixation was calculated and nodules were found to contain N that was predominantly derived from N_2-fixation. Leaves of both species had derived a slightly higher % of their N from N_2-fixation than the roots, independent of the amount of ammonium sulphate applied. When different amounts of ^{15}N-depleted ammonium sulphate were used, significant differences in atom % ^{15}N of leaves, roots and nodules. These findings led to the use of ^{15}N-depleted ammonium sulfate in field studies to calculate the % N derived from N_2-fixation by legumes.

* N_2-fixation of intercropped cowpea as affected by plant density.

In many parts of the world more than one crop is grown simultaneously at the same site. Often, a legume is intercropped with a non-legume such as corn. However, not much is known about N-cycling or N-dynamics in such systems. A field experiment was conducted to study the effect of corn density on N_2-fixation of intercropped cowpea. Row spacing was varied and the N_2-fixation of the legume was measured using ^{15}N-depleted $(NH_4)_2SO_4$. Preliminary results indicate that the total yield/ha and total N/ha for corn and cowpea decreased with increasing row spacing. N_2-fixation decreased significantly when the spacing between the rows was increased. Intercropped cowpea showed a significant decrease in yield and total N as compred with monocropped cowpea. The % N derived from N_2-fixation, however, increased in intercropped cowpea.

These results support our findings in the greenhouse that ^{15}N-depleted ammonium sulphate can be used in field studies for assessing N_2-fixation.

* Establish whether nitrogen can be transferred from a legume to an accompanying non-legume by mycorrhizae.

Vesicular arbuscular mycorrhizal (VAM) fungi are known to facilitate nutrient uptake for plants. Furthermore, it has been shown that VAM fungi are able to transfer nutrients such as P and CO_2 compounds from one plant to another through hyphal connections.

It was hypothized that besides CO_2 and P, N also can be transferred through hyphae. The split-root technique was employed to test this hypothesis. Roots of soybeans were split between two pots and in one of the two pots, corn was also planted. Half of the pots in which corn and soybean were grown together were inoculated with VAM-fungi. Pots having only roots of soybeans received highly enriched ^{15}-$(NH_4)_2SO_4$.

Corn plants inoculated with VAM-fungi showed significantly higher atom % ^{15}N than the non-inoculated plants. These findings suggest that fixed nitrogen from legumes may be transferred to non-N_2-fixing plants through VAM fungi.

* Competition for nitrogen between N_2-fixing and non-N_2-fixing trees.

In many areas of the tropics, leguminous trees are used as shade trees in coffee and cacao plantations. To increase production, cacao and coffee trees are fertilized individually with N, P, and K. However, shade trees also profit from those fertilizer applications. The non-N_2-fixing tree, however, may more efficiently use the inorganic N than the N_2-fixing tree. To test this hypothesis, ^{15}N-depleted $(NH_4)_2SO_4$ was applied every four months for a one-year period to N_2-fixing shade trees, Inga jinicuil and coffee trees. It was found that I. jinicuil derived significantly more N from N_2-fixation when growing together with coffee trees than growing without coffee trees. This may indicate that coffee is more competitive for inorganic N than I. jinicuil or that excudates of coffee roots stimulate N_2-fixation.

* Effects of inoculating legumes with rhizobia and free living N-fixing bacteria on growth and BNF

A greenhouse experiment was conducted in the NifTAL greenhouse to examine the effect of Azospirillum and Azotobacter inoculation on the nodulation, shoot growth, and root proliferation of three legume species, Glycine max, Phaseolus vulgaris, and Vigna unguiculata. The experiment was also designed to detect possible interactions between the inoculation treatments, two P fertility levels, and Rhizobium strain competition.

A dual inoculation experiment.

Screened, limed Kuiaha soil was used at two P levels (100 and 400 kg/ha P). Azospirillim brasilense (SP107) and Azotobacter chroococcum cultures were cultured in try/yeast broth and diluted from 10^5 cells/ml, 1 ml/seed, for inoculation of pregerminated seedlings at the time of

planting. All plants were also simultaneously inoculated with 1 ml 10^7 cells/ml of the appropriate three-strain INLIT rhizobial mix. The strains were cultured separately and quantified to assure equal numbers in the mixture.

Treatments were -ASP-AZB, +ASP-AZB, -ASP+AZB, +ASP+AZB X 2 P levels, RCB design, with 5 replications. Harvests were one month after planting.

Assessing responses to P.

There were no positive responses to inoculation by the associative fixers; however, in Glycine there was a significant negative effect of Azospirillum inoculation on shoot weight.

There were significant responses to P by all species in terms of nodule mass, shoot dry weight, and shoot/root ratios. A random serological testing of the nodules revealed that in every case that one strain of the three-strain mixture for each species dominated (± 75%) nodule occupancy; TAL 102 in soybean, TAL 182 in bushbean, and TAL 173 in cowpea. This dominance occurred regardless of ASP/AZB inoculation or P status.

* Effect of plant spacing and nitrogen fertilization on nitrogen fixation by Phaseolus vulgaris intercropped with maize.

A study was run to determine the effects of planting geometry and levels of N-fertilization on BNF by dry beans intercropped with maize. Spacing treatments were: monocrops of maize or beans (2 plants/planting hole), alternating rows of beans and maize (2 plants/planting hole) or beans and maize intercropped (one bean and one maize plant/planting hole). Nitrogen was applied at 5, 50 and 100 kg/ha. Nitrogen fixation was measured using ^{15}N-enriched and ^{15}N-depleted labeled fertilizers as tracers.

Total plot biomass was highest in the corn monocrop, followed by the two intercrops, which were not significantly different from each other. Lowest biomass was obtained in the bean monocrop.

N level differences were significant for total combined bean plus corn biomass and corn biomass, but not for either total bean biomass or bean seed yield. Highest bean biomass was achieved in the bean monocrop and separate row intercrop, and lowest for the same-row hole planting. These results indicate that the particular bean cultivar used in the study exhibited maximum efficiency of grain production when intercropped. Similarly, when corn alone is considered, maximum biomass production occurred in the same hole planting followed by separate row intercropping. Lowest

biomass was achieved in the monocrop. As with beans, the results suggest that the local Mexican corn cultivar used in the study maximized production in an intercropped system. ^{15}N analyses and interpretation will be completed in 1985.

* The effects of rhizobia VAM fungus inoculation on establishment and growth of Prosopis pallida, Leucaena leucocephala, and Gliricidia sepium in an acid and neutral soil.

Seeds of the three species were planted in 6" dibble tubes containing Pro-mix potting mix and peat-based rhizobial inoculant and/or sand based VAM inoculant (5% by weight) containing spores and hyphal fragments of Glomus fasciculatus. Percent germination and initial seedling growth rates were determined. When seedlings were 30 cm high (at approximately 2 months of age), half were transplanted to the Kuiaha field site and half to the Pauwela site. While the soil at both sites is an Ultisol, Haiku clay series, the soil at Kuiaha has a pH of 4.5 while the soil of Pauwela has a pH of 7.0. Percent survival and height growth measurements were taken every two months. Results indicate no significant differences in height growth at either site between treatments after 6 months.

The report on the Rodale Conference is still in preparation and should be published in early 1985.

SOIL STRESS TOLERANCE

Objective

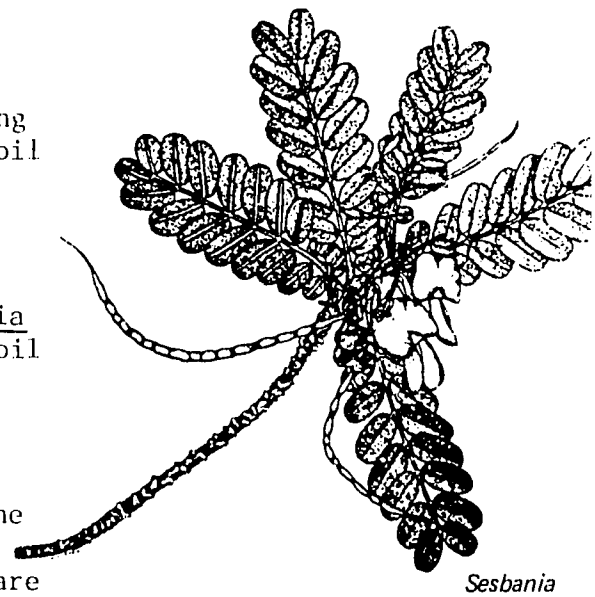
- to lower the cost of soil management, improve crop performance, and guide the choice of legumes for cropping systems by defining their tolerances to components of soil infertility

Accomplishments

Key experiments were conducted in assessing Sesbania and Leucaena species' tolerance to salt, an important soil stress condition affecting many areas of the world.

Research

Various species of Sesbania are found throughout the tropics. Several, such as Sesbania grandiflora and S. sesban are recognized as being fast-growing trees that are



Sesbania

good sources of firewood. Other species are less well known. One species, Sesbania bispinosa, is used as a green manure in India to reclaim land affected by high salinity and alkalinity. It would be useful to determine the extent of this species' salt tolerance as well as that of other Sesbania species. The purpose of this experiment was to screen eight seedlots of Sesbania species for NaCl tolerance. The design of the experiment was a complete randomized block design with two replications and four treatment levels of NaCl: 1 mM, 50 m M, 100 mM, and 200 mM.

SCREENING OF SESBANIA SPECIES FOR NaCl TOLERANCE

Species	Abbreviation	Origin	Scarification	Annual/ Perennial
S. sesban	SB1	Kenya	40 minutes	P
S. sesban	SB13	Egypt	20 minutes	P
S. grandiflora	GL17	India	5 minutes	P
S. pachycarpa	PC2	Senegal	60 minutes	A/P
S. emerus	XP	Peru	60 minutes	A
S. rostrata	RS1	Senegal	60 minutes	A
S. exasperata	EX3	Surinam	40 minutes	A
S. bispinosa	BA6	India	20 minutes	A

Seed for eight seedlots of Sesbania was obtained from Dale O. Evans of the University of Hawaii, scarified in sulfuric acid, and germinated in sterilized sand. Information concerning the seeds' country of origin, the plants' length of scarification is summarized in Appendix A, Graphs 1 - 4.

Two weeks after the seeds were planted, the young seedlings were transferred to 400 l tanks filled with nutrient solution and covered with styrofoam sheets with holes cut in them. Six plants of each species were suspended in each tank, with all eight lines present in each tank. NaCl was added to the tanks four days later in increments of 50 mM per day until the desired levels were obtained. The pH of the nutrient solution was maintained at about 6.5.

Twenty-five days after being transplanted into the tanks, the plants were harvested. The stems were cut next to the styrofoam lid and the shoots and roots were dried separately at 70 °C for 48 hours. The total dry weights of the roots and shoots of each species in each tank were obtained, the means of which are presented in graphs 1 - 4, Appendix A. These total dry weights are based on the weights of the plants that survived until harvest. However, at harvest, about half of SB13 in 200 mM NaCl had died. Therefore, the data given for SB13 may be misleading. Also, at harvest, all of PC2 in 200 mM had died.

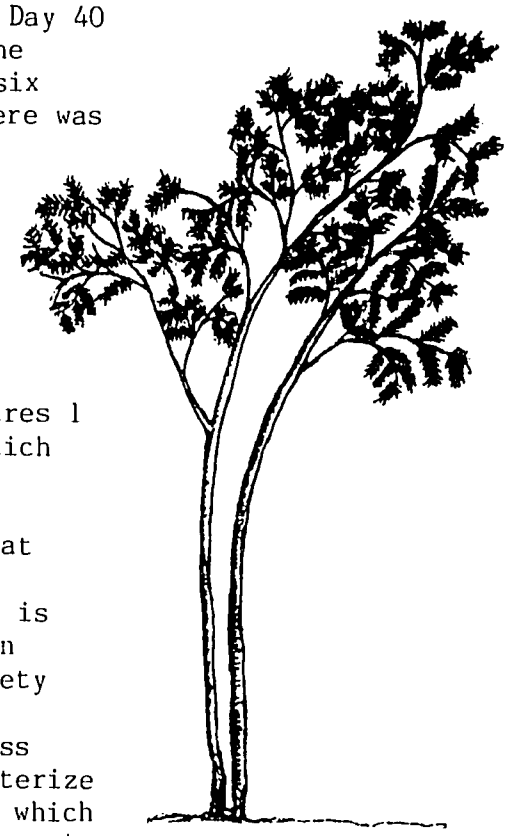
As can be seen from the graphs, most of the species exhibited a great deal of NaCl tolerance, especially up to levels of 100 mM. Even SB13, to which 200 mM induced 50% mortality, had only moderate growth reduction at 100 mM NaCl. PC2, Sesbania pachycarpa, was the only species found to be severly sensitive to high NaCl levels. GL17, Sesbania grandiflora, even appears to be somewhat halophytic, since shoot growth for both replicates was greater at 50 mM NaCl that at 1 mM, while at 100 mM NaCl growth was roughly equivalent to the control.

Leucaena leucocephala has been observed growing along the Hawaiian coast (NAS, 1977) and thus may be assumed to possess a certain degree of salt tolerance. A screening trial of two Leucaena varieties K8 and K64, was conducted using solution culture from October, 1983, until March, 1984, to determine the extent of this tolerance at high levels of salinity.

Seed was obtained from the University of Hawaii, scarified in sulfuric acid, and germinated in sand. Seedlings were inoculated with Rhizobium TAL 1145 and, when well nodulated, were transferred to 20 l buckets containing nutrient solution. The pH of the solution was maintained at about 6.5. A salt treatment of 6,000 mg NaCl/l solution was introduced two weeks later (on Day 14 on Figures 1 and 2) to two-thirds of the buckets. This level was doubled on Day 40 in half of the salt-treated buckets. The design of the experiment was complete randomized block design with six replications of K8 and four replications of K 64. There was one plant per bucket of nutrient solution.

Plant height was measured throughout the course of the experiment and is depicted in Appendix A, Figures 1 and 2. Contained in Appendix A, Table 7, are the final final dry weights of roots, stem, leaves, and nodules, and nodule number. The highest salt treatment (12,000 mg NaCl/l solution) killed several plants of each variety. Figures 1 and 2 are based on the mean heights of those plants which survived.

It is evident from Table 7 and Figures 1 and 2 that growth of both varieties of L. leucocephala is severely depressed at a NaCl concentration of 6,000 mg/l (which is equivalent to 103 mM NaCl, an EC of 12 mmhos/cm, and an osmotic pressure of 4.7 atm.). Overall, Leucaena variety K64 also appears less vigorous than K8, since with no addition of NaCl its final biomass is significantly less than that of K8. Further work is being done to characterize the tolerance of this species to lower levels of NaCl, which are more typically tolerated by legumes.



Leucaena

ECONOMICS OF BNF

Objective

- to devise and test appropriate methodology for measuring the economic benefits of the use of inoculated legume crops in farming systems of developing countries.

Accomplishments

Kilkenny's master's thesis, titled "An Economic Asessment of Biological Nitrogen Fixation in a Farming System of Southeast Minnesota," which presents the initial model of a farming system incorporating cropping, livestock, and household systems, was successfully defended. Several articles based on this thesis are being prepared by Kilkenny and Welsch.

A new research assistant, Keith Fuglie, was recuited. A small grant was obtained from AID/S&T/AGR to fund field work by Fuglie.

Fuglie was posted to Thailand in early October, 1984, under the auspices of the National Research Council of Thailand. His expected date of return to the University of Minnesota is September, 1985. Fuglie is analyzing the Farming Systems Project, Faculty of Agriculture, Khon Kaen University (KKU-FSP).

APPENDICES

APPENDIX A. Tables, Graphs, and Charts

TABLE 1.

"A" TESTS SENT AND RETURNED IN 1984

NETWORK	"A" TESTS SENT	"A" TESTS RETURNED
Asia	106	81
Africa	6	1
Latin America	26	11
Middle East	9	2
TOTAL	147	95

TABLE 2. Compiled data from International Network of Legume inoculation trials.

Vigna radiata

Data received at NifTAL - 19 experiments
Experiments in combined analysis - 15
Experiments withheld - 4
Experiments having significant inoculation effects - 3
Experiments having significant N source effects - 3
Treatment x Experiment interaction found - yes

Vigna mungo

Data received at NifTAL - 14 experiments
Experiments in combined analysis - 8
Experiments withheld - 6
Experiments having significant inoculation effects - 5
Experiments having significant N source effects - 5
Treatment x Experiment interaction found - yes

Vigna unguiculata

Data received at NifTAL - 7 experiments
Experiments in combined analysis - 5
Experiments withheld - 2
Experiments having significant inoculation effects - 1
Experiments having significant N source effects - 0
Treatment x Experiment interaction found - no

Arachis hypogaea

Data received at NifTAL - 9 experiments
Experiments in combined analysis - 8
Experiments withheld - 1
Experiments having significant inoculation effects - 2
Experiments having significant N source effects - 0
Treatment x Experiment interaction found - no

Cajanus cajan

Data received at NifTAL - 9 experiments
Experiments in combined analysis - 5
Experiments withheld - 4
Experiments having significant inoculation effects - 0
Experiments having significant N source effects - 0
Treatment x Experiment interaction found - no

Phaseolus vulgaris

Data received at NifTAL - 7 experiments
Experiments in combined analysis - 6
Experiments withheld - 1
Experiments having significant inoculation effects - 2
Experiments having significant N source effects - 0
Treatment x Experiment interaction found - no

Lens culinaris

Data received at NifTAL - 17 experiments
Experiments in combined analysis - 9
Experiments withheld - 8
Experiments having significant inoculation effects - 4
Experiments having significant N source effects - 4
Treatment x Experiment interaction found - yes

Cicer aretinum

Data received at NifTAL - 20 experiments
Experiments in combined analysis - 14
Experiments withheld - 6
Experiments having significant inoculation effects - 7
Experiments having significant N source effects - 4
Treatment x Experiment interaction found - yes

TABLE 3. INTERNATIONAL NETWORK OF INOCULATION TRIALS
SUMMARY OF ALL SHIPMENTS - BY COUNTRY

COUNTRY	BAGS OF INOCULANT
Argentina	1
Australia	5
Bangladesh	45
Benin	4
Brazil	16
Burma	12
Burundi	400
Chile	8
China	4
Colombia	1
Dominican Republic	20
Ecuador	2
Egypt	10
Ethiopia	2
Fiji	5
Ghana	9
Guatemala	15
Haiti	7
Honduras	5
India	862
Indonesia	41
Jamaica	10
Madagascar	400
Malaysia	3
Mexico	26
Mozambique	200
Nepal	34
Nigeria	9
Pakistan	591
Peru	30
Philippines	134
Rwanda	43
Saudi Arabia	6
Senegal	2
Sri Lanka	12
Sudan	3
Taiwan	4
Thailand	2
Turkey	14
Uganda	6
USA	106
Vietnam	10
Virgin Islands	3
West Indies	1
TOTAL	3,121

TABLE 4. INTERNATIONAL NETWORK OF INOCULATION TRIALS
SUMMARY OF ALL SHIPMENTS - BY SPECIES

SPECIES	BAGS OF INOCULANT
Acacia auriculiformis	7
Albizia gummifera	1
Albizia lebbek	1
Arachis hypogaea	116
Cajanus cajan	135
Calliandra calothyrsus	15
Centrosema pubescens	10
Cicer arietinum	169
Crotolaria juncea	1
Cyamopsis tetragonoloba	1
Desmodium intortum	8
Desmodium uncinatum	1
Dolichos lablab	2
Erythrina species	4
Glycine max	144
Glycine wightii	4
Inga species	4
Lens culinaris	54
Leucaena leucocephala	93
Medicago sativa	12
N.F.T.	21
Phaseolus vulgaris	55
Pisum sativum	88
Prosopis	1
Prosopis chilensis	2
Prosopis juliflora	2
Psophocarpus tetragonolobus	5
Sesbania	3
Single strain	68
Siratro	1
Sterile	1,606
Stylosanthes gracilis	1
Stylosanthes guianensis	23
Tephrosia vogelii	5
Trfolium alexandrinum	6
Trifolium species	3
Vicia faba	12
Vigna mungo	10
Vigna radiata	342
Vigna unguiculata	73
TOTAL	3,121

TABLE 5. CULTURES REQUESTS FILLED

1984 CULTURE REQUESTS

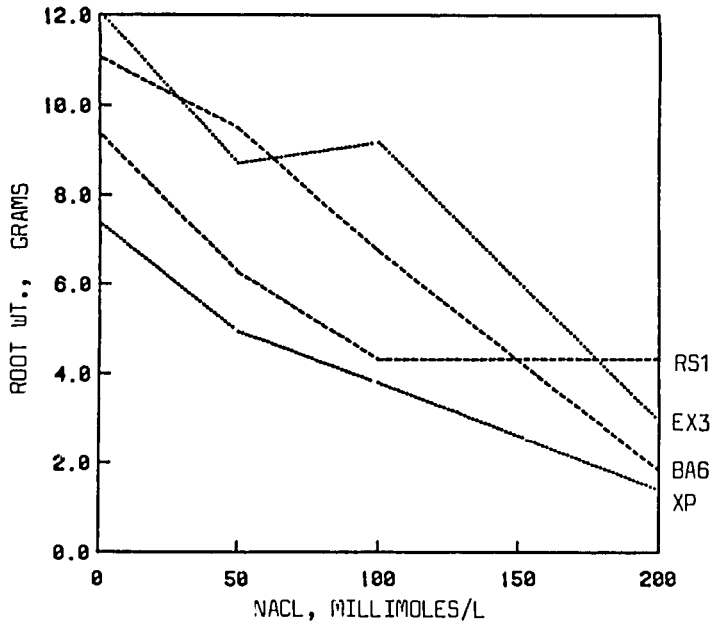
COUNTRY		NUMBER OF REQUESTS	NUMBER OF CULTURES
Australia		1	2
Bolivia		1	8
Brazil		1	11
Colombia		2	11
Costa Rica		1	24
Czechoslovakia		1	5
Dominican Republic		2	19
Egypt		1	4
El Salvador		1	3
England		1	7
Ethiopia		1	3
Ghana		1	2
Guatemala		1	6
Haiti		4	25
India		14	119
Indonesia		2	16
Japan		2	9
Malaysia		1	3
Mexico		1	11
Morocco		1	2
Pakistan		1	26
Panama		1	5
Saudi Arabia		1	4
Senegal		1	14
South Africa		1	3
Sri Lanka		2	22
Sudan		4	57
Thailand		1	6
USA		12	26
Venezuela		3	43
Virgin Islands		2	15
West Indies		1	5
Zaire		1	8
Zambia		2	26
TOTAL	34	73	583

TABLE 6. ANTISERA REQUESTS FILLED

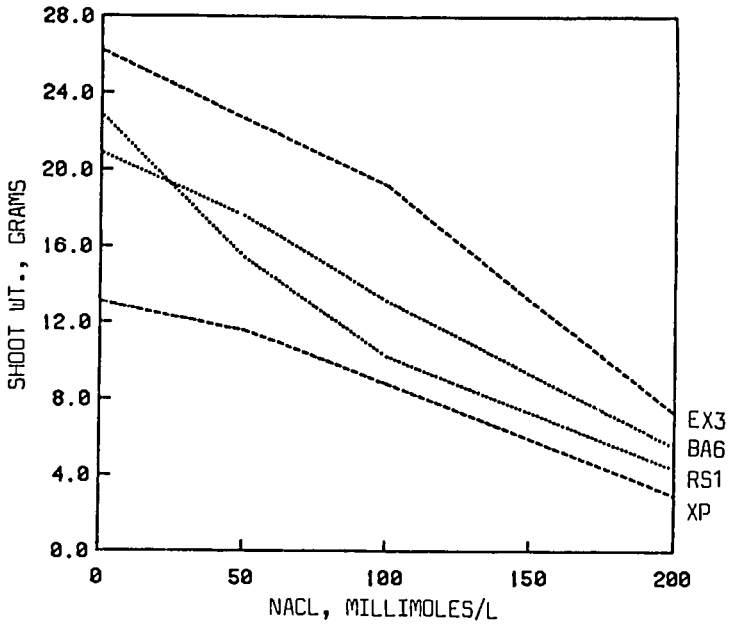
1984 ANTISERA REQUESTS

COUNTRY	ANTISERA FOR	NUMBER OF REQUESTS
Egypt		
	TAL 102	2
	TAL 377	2
	TAL 379	1
	TAL 638	3
	TAL 634	3
	TAL 640	3
	TAL 1397	1
	TAL 1399	1
	TAL 1400	1
	TAL 1402	1
India		
	TAL 169	2
	TAL 173	1
	TAL 209	1
	TAL 420	1
	TAL 441	1
	TAL 480	1
	TAL 569	1
	TAL 620	1
	TAL 658	1
	TAL 1000	1
	TAL 1127	1
	TAL 1132	1
	TAL 1148	5
Mexico		
	TAL 22	1
	TAL 102	1
	TAL 173	1
	TAL 182	1
	TAL 377	1
	TAL 379	1
	TAL 1376	1
	TAL 1383	1
Sudan		
	TAL 102	2
	TAL 182	2
	TAL 309	1
	TAL 377	1
	TAL 379	2
	TAL 620	2
	TAL 634	2
	TAL 638	1
	TAL 640	1
	TAL 1000	2
	TAL 1148	1
Turkey		
	TAL 102	1
	TAL 182	1
	TAL 309	1
	TAL 377	1
	TAL 379	1
	TAL 634	1
	TAL 638	1
	TAL 640	1
	TAL 1376	1
	TAL 1383	1

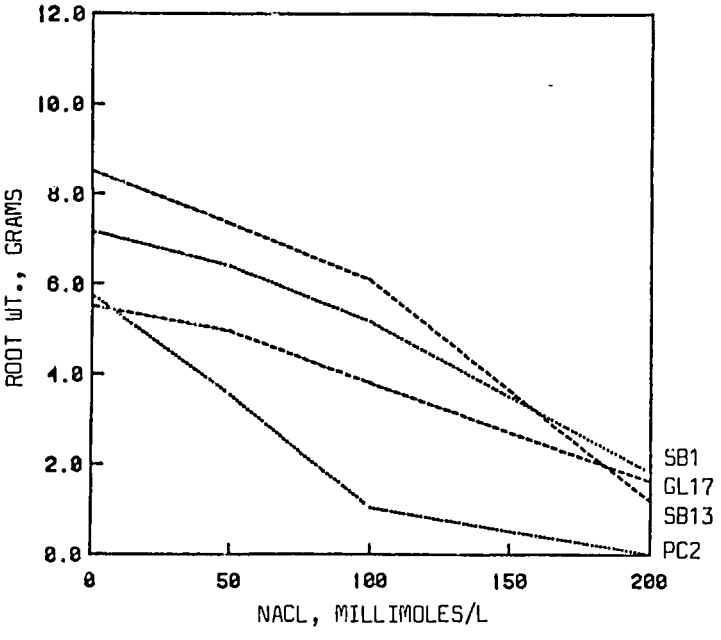
A TOTAL OF 393.8 ML OF ANTISERA WERE DISRIBUTED



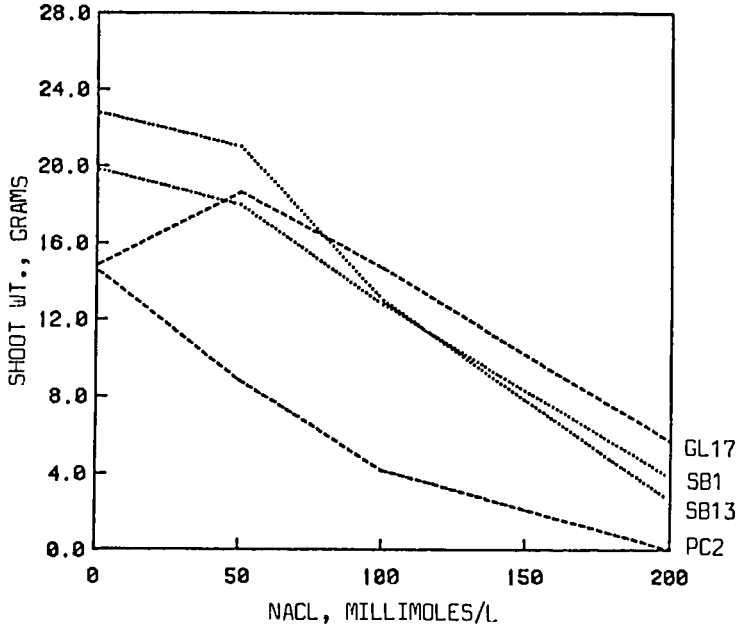
GRAPH 1. Root growth response to salinity for 4 annual lines of Sesbania.



GRAPH 2. Shoot Growth response to salinity for 4 annual lines of Sesbania.



GRAPH 3. Root growth response to salinity for 4 perennial lines of Sesbania.



GRAPH 4. Shoot Growth response to salinity for 4 perennial lines of Sesbania.

TABLE 7. Effect of NaCl on growth parameters of two varieties of *Leucaena leucocephala*

	NaCl conc.	Root wt.	Shoot wt.	Leaf wt.	Nodule wt.	Nodule no.	# surviving plants
	mg/l	-g-	-g-	-g-	-g-		
K8	0	12.885	3.978	5.613	0.959	481	6
	6000	2.508	0.598	0.882	0.137	75	6
	12000	0.891	0.195	0.043	0.020	31	3
K64	0	6.565	1.323	3.483	0.624	520	4
	6000	0.475	0.098	0.090	0.022	31	4
	12000	0.609	0.141	0.035	0.016	40	2

FIGURES 1 & 2

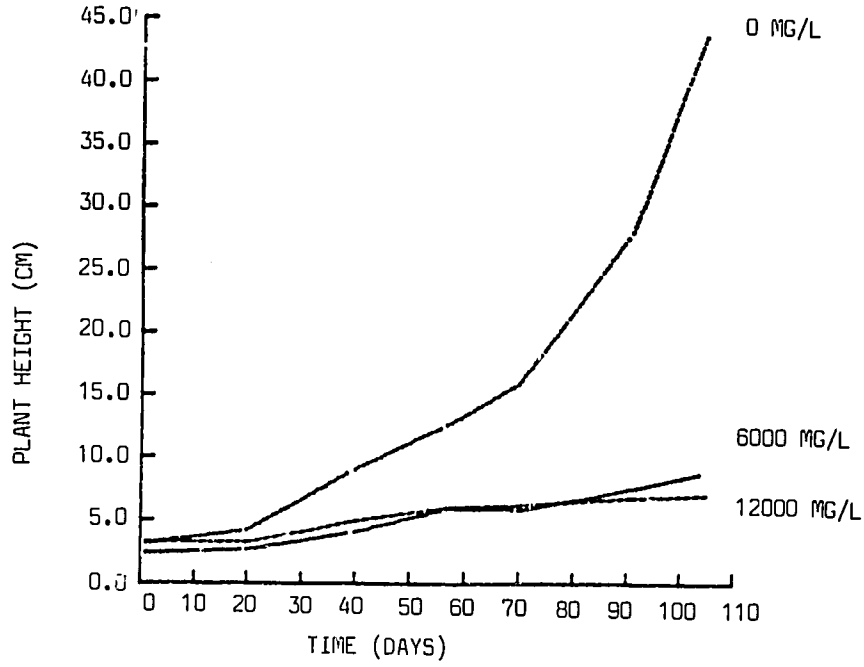


FIGURE 1. Plant Height of K8 with time at three levels of NaCl.

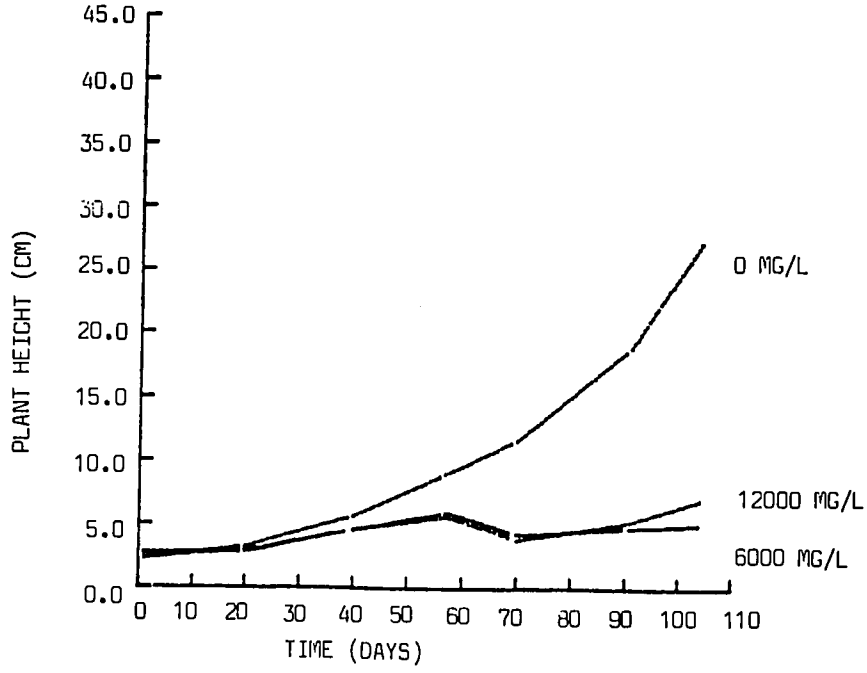


FIGURE 2. Plant height of K64 with time at three levels of NaCl.

APPENDIX B. 1985 Work Plan

OBJECTIVE 1 - Germplasm

1. Biochemical, intrinsic antibiotic resistance and serological properties of fast-growing rhizobia nodulating nitrogen-fixing tree species. (Somasegaran and Hoben)
2. Germplasm maintenace (Somasegaran)
3. Host-Rhizobium interactions and competition for nodulation in 3 cultivars of Phaseolus vulgaris. (Somasegaran and Hoben, Also comes under Objective 4)

OBJECTIVE 2 - Antiserum

1. Antiserum bank maintanance (Hoben)

OBJECTIVE 3 - INLIT

1. Publish experiment station bulletin on INLIT. (Davis)
2. Continue supply and service to BNF workers in LDCs. (Davis and Network Coordinators)
3. Provide TA to missions/HCs upon request. (host country)
4. Compilation, summarization, and analysis of INLIT data. (Davis, Cady, Chan)
5. Continue to conduct INLIT experiments on Maui. (Davis)

OBJECTIVE 4 - Inoculants

1. Influence of inoculation rates on the nodulation, dinitrogen fixation (C_2H_2 reduction) and competition of Rhizobium strains on chickpea (Cicer arietinum) in soils free of native chickpea rhizobia. (Somasegaran and Hoben)
2. Inoculum production maintenance. (Somasegaran)
3. Support for Zambia Project. (Singleton)
4. Multistrain legume inoculants: Competition of Rhizobium strains during growth in the liquid medium, in the inoculant, on the inoculated seeds and during nodulation. (Somasegaran and Hoben)
5. Characterization of a multi-strain Rhizobium inoculant for nitrogen-fixing trees.

OBJECTIVE 5 - Crop Systems

1. N-mangement and conservation under different soil stresses. (Singleton and van Kessel)
2. Assessment of N_2-fixation by trees. (Singleton and van Kessel)
3. N_2-competition between trees. (van Kessel and Roskoski)
4. Strain specific carbon partitioning to nodules on soybean roots nodulated by more than one strain of Rhizobium. (Singleton and van Kessel)
5. Publish Proceedings of Rodale Workshop. (Roskoski)
6. Effects of nitrogen and spacing configuration on nitrogen fixation by Phaseolus vulgaris intercropped with corn. (Roskoski)
7. A greenhouse study of nitrogen mineralization, uptake, and denitrification from different legume residues. (Roskoski)

OBJECTIVE 5 (continued)

8. Nitrogen balance of a soybean field study, taking account of N inputs via rain, irrigation water, beginning and ending soil N levels, nitrogen losses via leaching and denitrification, N uptake from residues by subsequent corn crop. (Roskoski and van Kessel)
9. The effect of some soil stress factors on the nodulation process of soybean. (Singleton and van Kessel)

OBJECTIVE 6 - U.C. Davis Subcontract

1. Determine the tolerances for salt, acid and phosphate in various legumes.
2. Characterize mechanisms of acid and aluminum tolerance in rhizobia and symbiotic systems.
3. Relate tolerance of a strain to the conditions from which it was isolated.
4. Validate lab and greenhouse screening methods as indicators of stress tolerance in the field.

OBJECTIVE 7 - Economics of BNF

1. Develop a special model of farming system under study, solve the model, and test sensitivity of model to various changes. (Welsch)
2. Thailand cooperative research on farm earnings of the introduction of peanuts grown on residual soil moisture in the early dry season after wet season rainfed paddy rice in a rainfed paddy rice farming system in Khon Kaen Province of Northeast Thailand. (Welsch)

OBJECTIVE 8 - BNF Resource Center (Thailand)

1. Continue the regional research programs and development of close ties with selected institutes in each country of the region with a view to establishing a BNF Resource at the national level. Continue the internship program.
2. Continue research/technical support program and information distribution, with strengthening of inter-institution linkages.
3. Provide the following training services: conduct extension training course in Legume/Rhizobium inoculation and use; offer a 3-week Inoculum Production Course (based on the model of NifTAL's 1984 course); and continue distribution of training materials, with increasing emphasis on extension-type information.

OBJECTIVE 9 - Training & Communication

1. Training workshop for extension workers in Bangladesh.
2. Travel, training-related
3. Intern participants
4. Graduate student sponsorship
5. Publications

APPENDIX C. Bibliography of NifTAL Research

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* Singleton, P.W., H.M. Abdel Magid, and J.W. Tavares, 1984. The Interaction Between Soil N Availability, Rhizobial Strain Effectiveness and N_2 Fixation by Soybean. World Soybean Reseach Conference III Abstracts. Ames, Iowa.

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APPENDIX D. 1984 Research Abstracts

Published Papers

* Beck, D.P., and D.N. Munns, 1984. Phosphate Nutrition of Rhizobium spp. Appl. Envir. Micro. 47(2):278-282.

This study was conducted to determine the behavior of 40 strains from six species of Rhizobium in liquid defined media containing orthophosphate at levels likely to be encountered naturally, ranging from the high concentrations expected in nodules and artificial media to the low concentrations of soil solutions. Storage capacity in strains with high levels (2 mM) of P and ability to utilize this stored P for growth after transfer to low levels (0.06 uM) of P varied with each strain. Storage varied from about 1 to 2% P (dry weight) for all strains, with the number of generations supported dependent on the quantity of P stored and on the utilization efficiency. The ability to store P at high levels is probably less important than the uptake and utilization efficiency of P supplied at low levels. Strains varied greatly in tolerance to low levels of P maintained in solution by an iron oxide buffering system. Differences in growth rate at low levels of P were large enough to be agronomically important.

* Bohlool, B.B., Kosslak, R., and R. Woolfenden, 1984. The Ecology of Rhizobium in the Rhizosphere: Survival, Growth and Competition. In C. Veeger and W.E. Newton (Eds.) Advances in Nitrogen Fixation Research. Martinus Nijhoff/ Dr. W. Junk Publ., The Hague, p. 287-293.

Quantitative ecology of desired strains of Rhizobium in soil, rhizosphere and nodules can be studied directly using strain-specific fluorescent antibodies. Competition among strains for nodulation of the appropriate host is a complex process involving strain/strain, as well as, strain/host interactions. This paper discusses the rhizosphere competence of different strains of R. japonicum and R. leguminosarum in the rhizosphere of soybeans, peas and corn in relation to nodule occupancies of each strain on the host. Evidence is presented on how the host plant may orchestrate the selection of one strain over another in the early stages of the infection process.

* Burton, J.C., 1984. Legume Inoculant Production. NifTAL, Hawaii, 50 p. No abstract.

* Cunningham, S.C., and D.N. Munns, 1984. The Correlation Between Extracellular Polysaccharide Production and Acid Tolerance in Rhizobium. Soil Sci. Soc. Am. J. 48:1273-1276.

Rhizobial isolates from six host genera (Cicer, Phaseolus, Leucaena, Lens, Melilotus, and Trifolium) were screened for ability to colonize successively more acidic agar plates. Sufficient isolates from Cicer and Phaseolus were screened to show that relative tolerance to acidic conditions was normally distributed. Extracellular polysaccharide (EPS) was isolated by ethanol precipitation from 10-d-old strains. Acid-tolerant isolates from Cicer, Phaseolus, Leucaena and Melilotus produced significantly more ($P < 0.001$) EPS than did those which were acid sensitive. The differences between isolates from Trifolium were significant at the $P < 0.05$ level. No significant difference in EPS production was found among the 8 isolates from Lens. The correlation between EPS production and acid tolerance was tested further with EPS isolated from 20 strains of Rhizobium phaseoli. The quantity of EPS produced varied from 18 to 75 mg 100 mL culture, was normally distributed, and albeit with an r^2 value of only 0.331. It is postulated that EPS could modify the microenvironment of the rhizobia and so decrease the stresses induced by an acid soil.

* Cunningham, S.C., and D.N. Munns, 1984. Effects of rhizobial extracellular polysaccharide on pH and aluminum toxicity. Soil Sci. Soc. Am. J. 48:1276-1279.

A positive correlation has been demonstated between the ability of Rhizobium strains to colonize acidic agar surfaces and their production of extracellular polysaccharide (EPS) in solution culture at pH 7.0. This set of experiments examined the effects of EPS on solution pH levels and Al activity, two common growth-limiting factors in acid soils. Samples of EPS from each of 20 previously acid-screened Rhizobium phaseoli isolates were titrated under controlled conditions and a buffering capacity was calculated. In addition, EPS from all strains were compared for ability to chelate aluminum by sodium saturation followed by equilibration against an aluminum - phospate solid phase. Total aluminum ofthe

digested samples was measured by atomic adsorption spectrophotometry. The buffering ability of the EPS varied considerabley between strains but was not correlated with the acid tolerance of th strain from which each was isolated. Similarly, while there were significant differences in the ability of the EPS to chelate aluminum, these were not correlated with acid tolerance. Acid tolerance did not correlate with EPS production weighted for either buffering capacity of the EPS or its ability to chelate Al. Thus, while the amount of ethanol-precipitable EPS from different rhizobial strains varied significantly in these two capacities, rhizobial EPS does not appear to confer acid tolerance on a strain by means of buffering pH or aluminum levels. Other possible causes of the observed relationship between acid tolerance and EPS production should be examined. These could include a role in increasing solution phosphate levels and/or the necessity of increased EPS for binding to plant lectins in an acidic environment.

* Davis, R.J., Halliday, J. and F.B. Cady, 1984. Preliminary Data from Worldwide Legume Inoculation Trials. In C. Veeger and W.E. Newton (Eds.) Advances in Nitrogen Fixation Research. Martinus Nijhoff/Dr. W. Junk Publ., The Hague, p. 333.

INLIT is a program initiated in 1980 which constitutes a network of cooperators which are either individual research scientists or institutions. These cooperators are located in developing countries and conduct legume inoculation trials. Cooperators are supplied a standard experimental design, supplies such as an inoculation package (seed if necessary), consultation, training and data analysis. Results from 110 trials has been reported and indicate that Glycine max gives the most consistent evidence of a response to inoculation, being directly associated with prior plantings and inoculation of the crop. However, trends are evident in other species indicating that when more locations have been reported factors will be identified which will indicate whether or not a response can be expected with these species.

* Eriksen, F.I., and A.S. Whitney, 1984. Effects of Solar Radiation Regimes on Growth and N2 fixation of Soybean, Cowpea, and Bushbean. Agron. J. 76(4):529-535.

Legumes are commonly grown in the tropics under reduced solar radiation due to dense cloud cover and shading from plantation or tall intercrops. This paper examines the growth and N2 fixation of several grain legumes common to the tropics on an Oxic Haplustoll in Hawaii. Plantings were made in April (warm season) and November (cool season) of soybean (Glycine max L. Merr.) 'Kahala'; cowpea (Vigna unguiculata Walp.) 'TVu 1190' (April) and 'TVu 4557' (Nov.); and bushbean (Phaseolus vulgaris L.) 'Burpee Tenderpod'. Polypropylene netting was used to obtain 27, 45, 70, and 100% of the incoming solar radiation through the entire crop cycle. Consistent shading effects were with the three legumes during both warm and cool seasons even though absolute yields were lower in the cool season due to day length and temperature sensitivity in soybean and cowpea. Nitrogen fixation parameters generally followed dry matter (DM) and seed yields. Total nodule activity (TNA) was very low in all the legumes at 27% sun. With the exception of bushbean at 27% sun, shading caused a 34% average reduction in DM and grain yields, vs. a 50% reduction in TNA, and a 76% reduction in nodule mass. Soybean TNA peaked 3 to 4 weeks after flowering, cowpea (cool season) TNA peaked at flowering, and bushbean TNA peaked slightly before or at flowering. There was a tendency for a delay (approx. 2 weeks) at 70, 45, and 27% sun in the final stages of TNA, for summer and cool-season soybean and bushbean. These delays were not noted for cowpea or cool-season bushbean. Of the three legumes, cowpea was the least shade tolerant. It produced more DM at full sun than either soybean or bushbean, but at 27% sun, DM yields were similar for all three crops. The response of soybean to shade was intermediate with no reduction in DM and grain yield at 70% sun. Bushbean was the least affected by shading; in fact, maximum yields were obtained at 70% sun. At full sun, their leaves were yellow and seed weight was low; this problem could be corrected by N fertilization.

* Halliday, J., 1984. Integrated Approach to Nitrogen Fixing Tree Germplasm Development. Rev. Bras. Sci. Agropec. (special volume), 21 p.

The performance of nitrogen fixing trees introduced to new environments depends on proper reconstitution of the symbiotic associations on which the trees rely for their nutrition. Thus selection strategies employed to identify adapted germplasm for particular sites must provide for three-way selection of seed, rhizobia, and mycorrhizae. Selected lines must then be multiplied before they can be deployed in varying types of development programs. Special problems

are faced in accomplishing these ends with virtually all nitrogen fixing trees. Results and experiences are described which emphasize the importance of parallel selection of plant germplasm and Rhizobium strains. In the case of VA mycorrhizae, effective symbioses can occur without specific inoculation. Methods for selecting and multiplying trees and their microsymbionts on a large scale are described and discussed.

* Halliday, J., 1984. Guidelines for Collecting the Root Nodules of Leguminous Trees. In P. Huxley, et al (Eds.) A Manual of Methodology for the Exploration and Assessment of Multipurpose Trees (MPTs). ICRAF, Nairobi. No abstract.

* Halliday, J., 1984. Methods for Assessing Rhizobia and VA Mycorrhizae for Multipurpose Trees. In P. Huxley, et al (Eds.) A Manual of Methodology for the Exploration and Assessment of Multipurpose Trees (MPTs). ICRAF, Nairobi. No abstract.

* Halliday, J., 1984. Principles of Rhizobium Strain Selection. In M. Alexander (Ed.) Biological Nitrogen Fixation. Plenum Publ., Corp. p. 155-171. No abstract.

* Halliday, J., 1984. Guidelines for Collecting the Root Nodules of Leguminous Trees. Nitrogen Fixing Tree Res. Reports Vol. 2. No abstract.

* Halliday, J., 1984. Register of Nodulation Reports for Leguminous Trees and Other Arboreal Genera with Nitrogen Fixing Members. Nitrogen Fixing Tree Res. Reports Vol. 2. No abstract.

* Halliday, J., and P. Nakao, 1984. Technical Note on the Germination of Leguminous Tree Seeds. Rev. Bras. Sci. Agropec (special volume).

Seeds of 33 leguminous tree species were tested for germination following mechanical or chemical scarification. The treatments gave germination of over 50% for 28 of the species. Mechanical or chemical scarification of about three times the number of seeds needed for an experiment with leguminous tree species is recommended as a rule of thumb when the germination rate of the seeds is in doubt.

* Halliday, J., and P. Somasegaran, 1984. The Rhizobium Germplasm Resource at NifTAL Catalogue of Strains. NifTAL Project, 94 p. No abstract.

* Kilkenny, M.R., 1984. An Economic Assessment of Biological Nitrogen Fixation in a Farming System of Southeast Minnesota. M.S. Thesis, Univ. of Minn. 215 p.

* King, J.W., 1984. Large University Agricultural Projects - Communication Problems and Prospects. ACE Quarterly 67(1):19-24.

This paper discusses the problems of and prospects for international communication sections of large university agricultural projects. Roles of communication sections included media production, information dissemination, publication, library maintenance, and training. Most sections see themselves as linking mechanisms, translating and tailoring project content for identified special audiences. Problems were identified as "systems" problems (lack of funding, staff turnover, ill-defined audiences, lack of credibility, lapses in intraproject communication, limited interpretation of communication, and inability to reconceptualize roles) and "diffusion" problems (inadequate unsystematic structure, lack of openness, inadequate capacity, lack of reward, distance and lack of synergy). Prospects for communication sections reflected communication opportunities (systems approaches, network funding and emerging characteristics of future-oriented sections) and individual approaches (professionalization of communication, need to be proactive and continuing education).

* Munns, D.N., 1984. Coping with Soil Acidity in Legume Symbiosis. Australian Inst. of Agric. Sci. Occasional Pub. No. 12. No abstract.

* Roskoski, J.P., and T. Wood, 1984. Nodulation and Nitrogen Fixation by Five Species of Leguminous Trees Grown in Soil From Undisturbed and Disturbed Tropical Sites in Mexico. In C. Veeger and W.E. Newton (Eds.) Advances in Nitrogen Fixation Research. Martinus Nijhoff/ Dr. W. Junk Publ., The Hague, p. 357.

Leguminous trees that grow rapidly, fix nitrogen, and produce fuelwood and protein-rich foliage and

fruits hold potential for use in tropical areas. Many of the valuable characteristics of these plants depend, in part, on the formation of effective nitrogen-fixing symbiosis. Examined was the ability of tropical soils from different sites in Mexico to supply rhizobia to Acacia pennatula Schl. and Cham., Albizia lebbek L., Enterolobium cyclocarpum Jarq., Gliricidia sepium Jarq., and Leucaeana leucocephala Benth. These species were planted in soil from a primary rain-forest, two slash-burn fields, two pastures, a secondary forest and two eroded sites. Plant height and biomass, and nodular biomass and specific activity were determined on 2 and 6 month old seedlings. The latter were also used to establish $^{15}N_2/C_2H_2$ conversion ratios. Overall, we found that even soils from adjacent areas varied markedly in their ability to support tree legume growth and nodulation, and that conversion of tropical forests to other land uses increased the diversity and abundance of rhizobia specific of leguminous trees.

* Singleton, P.W. and B.B. Bohlool, 1984. The Effect of Salinity on Nodule Formation by Soybean. Plant Physiol. 74:72-76.

A split-root growth system was employed to evaluate the effect of NaCl on nodule formation by soybean. By applying the salinity stress and rhizobial inoculum to only one-half the root system the effects of salinity on shoot growth were eliminated in the nodulation process. Rhizobium colonization of root surfaces was not affected by the salt treatments (0.0, 26.6, 53.2 and 79.9 mM NaCl). While shoot dry weight remained unaffected by the treatments, total shoot N declined from 1.26 g N/pot at 0.0 mM NaCl to 0.44 g N/pot at 79.9 mM NaCl. The concentration of N in the shoot declined from 3.75% N (control) to 1.26% N at 79.9 mM NaCl. Nodule number and weight were reduced approximately 50% at 26.6 mM NaCl, and by more than 90% at 53.2 and 79.9 mM NaCl. Total nitrogenase activity (C_2H_2 reduction) declined in relation to nodule number and dry weight. Specific nitrogenase activity, however, was less affected by salinity and was not depressed significantly until 79.9 mM NaCl. Isolates of R. japonicum reisolated from nodules formed at 79.9 mM NaCl did not show increased nodulation of roots under salt stress compared to nodule isolates from normal media. The early steps in nodule initiation are, therefore, extremely sensitive to even low concentrations of NaCl. The sensitivity is not related to rhizobial survival and is probably due to the salt sensitivity of root infection sites.

* Singleton, P., and B.B. Bohlool, 1984. The Nodule Function and Nodule Formation in the Soybean-Rhizobium japonicum Symbiosis. In C. Veeger and W.E. Newton (Eds.) Advances in Nitrogen Fixation Research. Martinus Nijhoff/ Dr. W. Junk Publ., The Hague, p. 545.

Symbiotic nitrogen fixation may be adversely affected by saline environments. Described are three experiments that assess the salt sensitivity of: 1) Rhizobium as free living organisms; 2) soybean nodule function; and 3) soybean nodule formation. In addition, a split-root plant growth system is described which can be used to separate the effects of salinity stress on host yield potential from the effects of salinity on nodule processes.

* Somasegaran, P., Reyes, V.G., and H.J. Hoben, 1984. The Influence of High Temperatures on the Growth and Survival of Rhizobium spp. in Peat Inoculants During Preparation, Storage, and Distribution. Can. J. Micro. 30(1):23-30.

Gamma-irradiated peat was used to prepare inoculants for ten different species of tropical legumes and sent to cooperators in 14 cities in 13 tropical countries. Each cooperator received a package containing a maximum recording thermometer, plating media components, special instruction sheets and inoculants. Control experiments were pursued in the laboratory by exposing the various inoculants to 28, 37, and 46 degree C. Temperatures reached in the inoculants during their transportion varied from 26 degree C (Mexico) to 45 degree C (Kenya). Arrival time of the inoculants ranged from 6 days (Hissar, India) to 54 days (St. Augustine, West Indies). Although a total loss of viability was reported for the chickpea inoculant (Saudia Arabia) and a severe decrease in two others (bean and lentil inoculants in Kenya and Saudi Arabia, respectively), over 90% of the inoculants received had viable counts in excess of 1×10^9 cells per g moist peat. Laboratory data indicated that 28 degree C was optimal for multiplication to maximal numbers in excess of 1×10^9 cells per g moist peat. Six inoculants studied for long-term storage showed excellent quality for 24 weeks at 28 degree C. At 37 degree C there was comparable cell multiplication as at 28 degree C with 8 of the 10 strains, only during the first week 46 degree C was lethal for all strains. Large reductions in viable counts, observed during inoculant preparation when broth cultures are added to peat, may be attributed to a dilution undergone by the broth culture in the peat.

APPENDIX E. Participants

INTERN

NAME	COUNTRY	SPONSORSHIP S = NifTAL Shared T = NifTAL Total
Tomas Tello Reyes	Peru	S
Frank Sorwli	Ghana	T
Wilson Kagobo	Liberia	S
K. S. Jauhri	India	S
S. R. Hegde	India	S
Hu Zheng Jia	People's Republic of China	S
Lal Senayake	Sri Lanka	S
Wichien Yongmanitchai	Thailand	S

Inoculant Production Course, Maui, December

A. E. El Shafie	Sudan	S
Arturo C. Espinoza	Mexico	S
Albrecht Glatzle	Morocco	S
Walt Graves	USA	S
Jeff B. Million	West Indies	S
Catharina Chou Pan	Brazil	S
Muhrizal Sarwani	Indonesia	S
Carlos G. Suarez	Dominican Republic	S
Wichien Yongmanitchai	Thailand	S
Mongi Zouaghi	Tunisia	S

Azolla Course, Thailand, January

Dedeh Hudaya Arief	Indonesia	T
S. Kannaiyan	India	T
Soetjipto Partohardjono	Indonesia	T
N. R. Vishwanath	India	T
Ruchyat Damanhuri	Indonesia	T
Sikander Ali	Pakistan	S
Naseer Mirza	Pakistan	S
Antonio E. Leviste	Philippines	S
Juan C. Bunoan, Jr.	Philippines	T
Celso R. Bersabe	Philippines	T
Charles de la Torre	Philippines	T

APPENDIX E. (Cont.)

NAME	COUNTRY	SPONSORSHIP S = NifTAL Shared T = NifTAL Total
Azolla Course - Thailand (Cont.)		
Abelardo Sorillo	Philippines	T
Romy Necesario	Philippines	S
Corazon Espinas	Philippines	S
Nirmala Gunapala	Sri Lanka	T
S. A. Kulasooriya	Sri Lanka	T
S. W. Abeysekera	Sri Lanka	T
Alan Moore	Australia	S
Sk. Md. Abdul Sattar	Bangladesh	S
Md. Abdul Majid	Bangladesh	S
Charles Van Hove	Belgium	S
Short Term Interns		
Taweesakdi Sesavej	Thailand	S
Awata Dalodom	Thailand	S
Pramual Setarat	Thailand	S
Somsakdi Surya	Thailand	S
Chavalvut Chainuvati	Thailand	S
Muhammad Majlis	Bangladesh	S
Muhammad Islam	Bangladesh	S
Faisal A. Chowdhury	Bangladesh	S
Muhammad Ali	Bangladesh	S
Tun Thein	Burma	S
Nuynt Wai	Burma	S
Shirley Smellie	Burma	S

Graduate Students

		Program	
Taufiqul Aziz	Bangladesh	Ph.D.	T
Shiva Chaudhary	Nepal	Ph.D.	T
Joshua Daniel	Sri Lanka	Ph.D.	T
Stephen Dowdle	USA	Ph.D.	T
Thomas George	India	Ph.D.	T
Wilson Kagabo	Liberia	M.S.	S
Ronnie Nyemba	Zambia	M.S.	S

APPENDIX F. Visitors at NifTAL

Visiting Scientist Program

Duane Bartholomew	UH Dept of Agron & Soils
Joshua Daniel	Kandy, Sri Lanka
Judith Bale	NAS, Washington, D.C.
Malcolm Bale	World Bank, Washington, D.C.
Bob Stevens	Kula Experimental Stn.
Ashok Alva	Univ. of Queensland, Australia
Louis H. Kuhn	USAID, Suua, Fiji
Ken MacDicken	WA
Paul Weatherly	Office of Energy, Washington, D.C.
Gordon Tsuji	IBSNAT Project, UH Manoa
Annette Chang	UH Dept of Agron & Soils
L. Chase	Honiara, Solomon Islands
Delane & Kay Welsch	Dept of Ag & Appl Econ, Univ. of Minn
John Gerdes	Maui, HI
M.S. Islam	BARI, Dhaka, Bangladesh
F.A. Choudhary	Bangladesh Agric Univ, Bangladesh
M.I. Ali	Bangladesh Agric Univ, Bangladesh
M.A. Khan Majlis	Bangladesh Jute Res Inst, Bangladesh
Wilson E. Kagabo	UH Manoa
Stephen Dowdle	UH Manoa
Philip Filner	ARCO Plant Cell Research, Dublin, CA
Kenneth Brooks	College of Forestry, Univ of Minn
John Davidson	Eucalytus & Forestry Services, Australia
Gary Evans	CSRS, USDA, Washington, D.C.
Alaides Ruschel	CENA, Sao Paulo, Brazil
Graeme Blair	Forage Research Project, NSW, Australia
Ray Gill	CSIRO, Australia
Tomio Tomiyasu	Sumitomo Forestry Corp, Tokyo, Japan
Toru Kobayashi	Sumitomo Forestry Corp, Tokyo, Japan
H. Shigemitsu	Sumitomo Forestry Corp, Tokyo, Japan
Y. Mori	Sumitomo Forestry Corp. Tokyo, Japan
C.T. Lee	College of Agric, Univ. of Guam
Wayne & Eleanor Freeman	IADS, Gatlinburg, TN
Hu Zheng-jia	Huazhong Agric. College, Wuhan, PRC
Kanok Rerkasem	Chiangmai Univ, Bangkok, Thailand
Sam Portch	IADS/BARC, Bangladesh
David Mackenzie	CSIRO, Canberra, Australia
Po Yang Lai	Hawaii Dept of Agric
Otto Soemarwoto	Inst of Ecology, Bandung, Indonesia
Zhiyuan Fan	Chinese Acad of Agric Sci, Beijing, China
Pilar T. Parmo	Ministry of Agric, Phillipines
Terd Charoenwatana	Khon Kaen Univ, Thailand
B.G. Cagauan	Dept of Agron & Soils, UH Manoa
Francisco H. Isaga	Leyte, Philippines
Percy Sajue	UPLB, Los Banos, Philippines
Josue Maestrado	Ministry of Agric, Philippines
Vingilio Villancio	FSSRI/Ministry of Agric, Philippines
Ly Tung	VISCA, Leyte, Philippines
Rebecco M. Santiago	VISCA, Leyte, Philippines
Danilo B. Palang	Abuyog Expt Stn, Leyte, Philippines
Eleodono N. Lapasanda	FSDP-EN, Leyte, Philippines
Antonio D. Jumadag	Samar, Philippines

Rodolfo Escalada	VISCA, Leyte, Philippines
Nelson M. Esguerra	VISCA, Leyte, Philippines
Nestor M. Gioria	VISCA, Leyte, Philippines
Gary K. Bullard	Woy Woy, Australia
John Whitten	USDA/CSRS, Washington, D.C.
J.A. Silva	Dept of Agron & Soils, UH Manoa
Anne Alvarez	Plant Pathology, UH Manoa
Albert Martinez	Plant Pathology, UH Manoa
Max McFadden	USAID/S&T/FNR, Washington, D.C.
Ananta Dalodom	Ministry of Agri & Cooperatives, Thailand
Chavalvut Chainuvati	Dept of Agri Extension, Thailand
Somsak Suriyo	Dept of Agri Extension, Thailand
Lawrence S. Hamilton	East/West Center, UH Manoa
Napoleon T. Vergara	East/West Center, UH Manoa
Jose Virgilio	Sur, Philippines
A. Ramos	Sur, Philippines
V. Kesavan	Univ of Papua, New Guinea
D. Inapero	DPI Forest Service, New Guinea
Joseph Parei	Cook Islands
Daniel E. Parreno	FSDC Area V, Philippines
Nga Tuakana	Mangaia, Cook Island
Jose Luis Delgado	Xalapo, Mexico
Don MacLaurin	Inst of Tech, B.C., Canada
V. Nair	Kenyatta Univ College, Nairobi, Kenya
P.K. Nair	Nairobi, Kenya
William S. Null	Tacoma, WA
Bikenibeu Paeniu	Agri Dept, Funafuti Island
Anau Manarangi	Rarotonga, Cook Islands
Bill Raynor	PATS, Ponape, FSM
Matairangi Purea	Agri Dept, Cook Islands
Wally Sanford	Agron & Soil Sci Dept, UH Manoa
D.J. Redgrave	Agron Dept, Colo State Univ, CO
Lannon Oldenborg	Nuku'alofa, Tonga
Dietrich Werner	Philippe Univ, Marsburg, W Germany
Upendra Singh	Dept Agron & Soils, UH Manoa
Bruce Trangmar	Soil Bureau, DSIR, New Zealand
T.C. Juang	FFTC/ASPAC, Taipei, Taiwan
A.L. Palafox	Mangilio, Guam
Doug Goguin	IFDC, Muscle Shoals, AL
Russell Yost	Agron & Soil Sci Dept, UH Manoa
R. Muniappan	Agri Expt Stn, Univ of Guam
Laye Kourouma	Agron & Soil Sci, UH Manoa
Thu-tak Tseng	Dept of Physics, Univ of Guam
James F. Parr	ARS/USDA, Beltsville, MD
Clement Mathieu	Faculty of Agron, Bujumbura, Burundi
Arlin Nicks	USDA - ARS, Durant, OK
L. Bascones	Maracax, Venezuela
T.S. Gill	USAID, Washington, D.C.
Daniel Dellach	INRA, France
Jeane Pierre Ndiaye	ISRA - CNRA, Bambey, Senegal
A.J. McGinnis	PARC, Islamabad, Pakistan
Raymond Kitchell	Alexandria, VA
Kaburungu Salvatore	Univ of Burundi
George H. Hargreaves	Utah State Univ, Logan, Utah
Vernon R.N. Chinene	Lusaka, Zambia

F.D. Whisler	Miss State Univ, MS
S. Panichapong	Land Dev Dept, Bangkok, Thailand
Marciano Rodriguez	CATIE, Turrialba, Costa Rica
Roel Oldeman	IRRI, Manila, Philippines
Joe T. Ritchie	USDA - ARS, Temple, Texas
Param Sivan	Nausori, Fiji
Wolfgang Gobel	ICARDA, Aleppo, Syria
Chatong Nammuang	Soil Sci Div, Bangkok, Thailand
F.H. Beinroth	Dept of Agron, Mayaguez, Puerto Rico
Dave Leslie	NZ Soil Bureau, DSIR, New Zealand
Bob Gavenda	Dept of Agron & Soils, UH Manoa
Steven Holzhey	Nat'l Soil Survey, USDA, Lincoln NE
P. Matt Cauley	Conservation Division, Lesotho, S Africa
Grant F. Walton	Dept of Env Res, Cook College, NJ
Barry Dent	Lincoln College, Canterbury, New Zealand
Henry Nix	CSIRO, Canberra, Australia
Ismail Abu Bakar	MARDI, Kuala Lumpur, Malaysia
Aurangzeb Khan	Soil Survey of Pakistan, Pakistan
Peter Sands	CSIRO, Canberra, Australia
Jorge Jonas	IDIAP, Panama
William Avilair	FONAIAP, Maracay, Venezuela
M. Akram Khan	Agri Engr, UH Manoa
J.A. Comerma	FONAIAP - CENIAP, Maracay, Venezuela
A. Berrada	INRA, Morocco
A.M. Osman	ACSAD, Damascus, Syria
Elmer E. Euring	Cornell Univ, Ithaca, NY
Thao Khamoui	College of Agri, Mangilao, Guam
James W. Jones	Univ of Florida, Gainesville, FL
J. Wayne Miskoe	Univ of Florida, Gainesville, FL
Pelihter Silbanas	Ponape, Marshall Islands
Shirley Smellie	Food Legumes Div (ARI), Pyinmana, Burma
Daw Nyunt Nyunt Wai	Central Farm, Burma
U Tun Thein	State Manager, Agri Corp, Moulmein, Burma
Charles Y.Yang	Thai Outreach Program, Bangkok, Thailand
Lal Senanayake	Dept of Agri, CARI, Peradeniya, Sri Lanka
Malee Suwana-Adth	SVITA Foundation, Bangkok, Thailand
Ray Carpenter	USAID, Mogedishu, Somalia
August P. van Gool	Univ of Leuvon, Heverlee, Belgium
Seth Beckerman	Washington State Univ, Pullman, WA
Donald R. Schmidt	Barat, Indonesia
Kauser Malik	NIAB, Faisalabad, Pakistan
Rakushausa Bilal	NIAB, Faisalabad, Pakistan
Thomas Bauer	Env Health & Safety, UH Manoa
Janet H. Cushman	Oak Ridge Nat'l Lab, Oak Ridge, TN
Mark A. Gerber	Pacific Northwest Lab, Richland, WA
Bjorn Ortenheim	Stockholm, Sweden
James L. Kuester	Arizona State Univ, Tempe, AZ
Duncan Macpherson	B.C. Inst of Tech, Vancouver, BC
R.J. Roughley	Dept of Agri, NSW, Australia
Art Bastmeyer	Geldrop, Netherlands
Douglas E. Eveleigh	Cook College, New Brunswick, NJ
Huang Zongdao	South China College of Tropical Crops
Huang Junzhong	South China College of Tropical Crops
Fang Zhonggen	South China College of Tropical Crops
Pong Yexiu	South China College of Tropical Crops

Visiting Citizens Program

Rick Koglin
Marilyn Harris
Sun Pani
John F. Bruce
G. Okazaki
Bert Meadows
Robert & Eleanor Lee
Myrna Pauscal
Brenda Kamaka
Samantha Dragon
Jeff Tavares
Laureen Tong
Jason Takayama
Lee Nakama
Gertrude Hoskinson
W.S. Hoskinson
Moses Kahalekau
Penny Kitchens
Rupert D. Rulloda
Nestor Cabanting
Jayma Martin
Gene Simpson
Mr. & Mrs. Russell L. Olson
Grant and Chalermsee Olson
Chuck Gray
Eric Herter
Cindy Seto
Michelle Wander
Paul C. Hanley
Alma Cooper
William Burt
Michelle Tai See
Rochelle Narimatsu
Gail Tai See
Kathleen Wilson
Mr. and Mrs. Robert Martinez
Sevath E. Boyum
John Southworth
Arthur Cribson
Ed and Karen Cohen
Bud and Lorraine Reynor
Ted Kurman
John C. Yuen
Andy Dudo
Sandra Sillapere
Bo Rodehn
Pete Marshell
Helen Lum
Harriet Uchigaki
Elaine Maraki
Helen Matsueda
Phyllis Strauske
Hermine Fleming
Cyrilla King
Hideo Talutani
Edward Matsueda
Daisy Alina
Walter Inouye
Motohisa Unemori
Bob and Judy Huglee
Ester Masako
Yoshio Sato
Monica Kyser
Hayano Sugaron
Arthur Harris
Kathleen Moser
Martin and Ruth Gutstein
D.R. Neil
Paul Ekern
Boyd W. Post
Ernest Regents
Wayne K. Satoshige
Rick Birken
Mike Caires
Philip Jones
Patrick Guth
Augie Salbosa
Paul G. Scowcroft
Andrew Namamura
Robbie Melton

APPENDIX G. New Course Outlines

Extension

OUTLINE: First Biological Nitrogen Fixation Research/ Extension Training Course held at BARI, Joydebpur, Bangladesh, 11-19 March 1984

Objectives:

1. To create awareness and understanding of the potentials and limitatins of Biological Nitrogen Fixation (BNF) in Bangladesh.

2. To upgrade the capabilities of local research/extension personnel in BNF technology.

3. To create a permanent working linkage between researchers and extension personnel.

4. To develop a training format for continuing similar training programs by local participants.

COURSE OUTLINE

Sunday, March 11

What is BNF? An Overview

1) N and its importance
2) benefits of BNF and its components
3) inoculation and successful use of BNF
4) future of BNF

Discussion

Strategy for a natinal BNF Program: An Overview

1) inoculum technology
2) farmer acceptance of technology
3) how to get complete technology

Current State of BNF in Bangladesh

Course Objectives and Orientation,
Presentation of Printed Materials

Participation baseline evaluation on BNF - Exam

Monday, March 12

Factors in Agricultural Production

Where BNF fits into production increase

1) Methods of yield improvement
2) Potential of BNF

Field Visit

1) Plot layout
2) Inoculation response
3) Nodule collection

Nitrogen Fixation Systems

1) How BNF works in legume/*Rhizobium*
2) Plant infection and nodule formation
3) Various legume/*Rhizobium* association
4) Diversity of systems
5) Other BNF systems (Azolla, BG, etc.)

Discussion

Rhizobium Bacteria

1) Isolation and identification
2) Specificity
3) Strain selection (criteria)

Rhizobium - Survival and Competition in Soil

1) *Rhizobium* nutrition
2) Soil chemical factors/stress

Rhizobium - Strain X legume cultivar reaction

Discussion

Tuesday, March 13

Azolla/Anabaena: An Overview

1) How does it work
2) Where it works
3) Current research
4) Benefits for Bangladesh
5) How it works in Bangladesh farming systems
6) Problems

Blue Green Algae

1) How it works
2) Where it works
3) Current research
4) Benefits for Bangladesh
5) How it fits into Bangladesh farming systems
6) Problems

Discussion

Inoculation: Overview of Tchnology

Inoculation Production

1) Processes and scale

Laboratory Demonstration on Production

Inoculum

1) Quality control, handling, and distribution

Hands-on Inoculation Process

Discussion

Wednesday, March 14

Soil Fertility: Problems and Practices in Bangladesh

1) General
2) As it may affect BNF

Water and Soil Management in Bangladesh

1) Practices and problems general
2) As they affect BNF

Discussion

Stress and Environmental Affects on Nodulation

1) Temperature
2) Drought
3) Pesticides

Discussion

Field Plot Design

1) To evaluate inoculation response
2) Plot layout and treatments ("A" expt)
3) Steps in experimental field trial
4) Data recording and management

Discussion

Hands-on Field Trial Establishment

1) Plot layout
2) Treatments (soil amendments)
3) Inoculation
4) Planting

Thursday, March 15

Data Interpretation of Field Results

Economic Benefits Through BNF Technology

1) What factors need to be considered
2) How to evaluate and interpret economic data

Research - Extension Interaction Necessary for Technology Transfer

Discussion

Extension - Farmer Interaction

Networking Within a Country to Maintain Information Exchange

1) Ideal
2) Actual in Bangladesh

Discussion

Crop Diversification: Cropping Systems With Legumes

1) In-region
2) In Bangladesh

Research/Extension Problem Demonstration

Critique of Demonstration

Distribution of Research/Extension Problems, Explanation, and Expected Results

Saturday, March 17 Field Trip to Mymensingh

Sunday, March 18

Presentation of Research/Extension Problem Demonstration by Each of 12 Groups and Critiques

Monday, March 19

Question/Answer Session with NifTAL the Original "What is BNF" Format: Answers supplied by Participant

BNF Resource Center for South and Southeast Asia

Future of BNF in Bangladesh: A Summation

1) Research needs and methodology
2) Who will cooperate
3) Linkages
4) Inoculum production
5) Technology transfer mechanism

Participant Evaluation - Exam
Course Evaluation by Participants

LEGUME INOCULATION PRODUCTION COURSE

OUTLINE: The methods and practices used for setting up low cost, small-scale legume inoculant production

COURSE OUTLINE

Monday, December 3

1) Opening remarks.
2) Overview - Legume inoculant production course.
3) History and development of the inoculant industry.
4) Orientation and site tour of NifTAL's production facility.
5) Exercise 5:
   a) Become familiar with the fermentor units.
   b) Examine air supply systems and pack and sterilize air filters.
   c) Prepare fermentors for operation.

Tuesday, December 4

1) Lecture: History and development of the legume inoculant industry
2) Exercise 1:
   a) Subculture rhizobia on YMA slants, reconstitute bead cultures in YM broth. Reconstitute lyophilized cultures on YMA plates.
   b) Culture rhizobia from slants and microorganism mixture in YMA + BTB +, YMA + Congo red and peptone glucose agar.
3) Exercise 2: Prepare agar plates with different carbon and nitrogen sources.
4) Exercise 5: Fill fermentor units.
5) Continue Exercise 2:
   c) Discuss growth media for Rhizobium.
   d) Make liquid media for fermentors.
6) Continue Exercise 1:
   c) Examine rhizobia and a mixture of microorganisms for cell onorphology using Gram stain and the simple carbol fuchsia stain.

Wednesday, December 5

1) Lectures:
   a) Rhizobia - microbiology, classification and use
   b) Selection of rhizobia for use in agriculture
2) Exercises 2-4: Inoculate media with different carbon and nitrogen sources using separate plates for:
   a) one fast-growing Rhizobium strain (TAL 380)
   b) one slow-growing Rhizobium strain (TAL 102)
3) Exercise 3: Start cultures (TAL 380 and TAL 120) in the following vessels:
   a) slants on Roux bottles.
   b) 250 ml Erlenmeyer flasks
   c) 500 ml filter flasks (connected pair)
   d) small glass fermentor
   e) 2800 ml Erlenmeyer flask

4) Exercise 4: Assemble small large glass fermentor units
5) Exercise 5: Set up small steel fermentor, test

6) Continue Exercise 4: Continue assembling glass fermentors, fill with liquid media, pretest and sterilize.
7) Continue Exercise 5: Retest fermentors for proper functioning. Learn trouble shooting procedures to overcome the most common problems.
8) Exercise 7:
   a) Examine several potential carrier materials e.g., peat, coal, charcoal. Discuss important qualities.
   b) Determine the moisture content of the carrier materials prior to processing. Discuss methods for drying carrier materials.

Thursday, December 6

1) Lecture:
   a) Selection of rhizobia for competitive ability
   b) Selection of rhizobia for environmental conditions
2) Exercise 3: Discuss and demonstrate inoculum transfer systems such as:
   a) Manual injection with a plastic syringe.
   b) Pouring from Roux bottles.
   c) Injection with a motorized syringe.
   d) Suction with a peristaltic pump.
   e) Pouring from connected filter flasks.
3) Exercise 5: Inoculate fermentors using methods demonstated in Exercise 3.
4) Exercise 7:
   a) Crush and grind carrier materials.
   b) Sieve carriers for granular types.
   c) Sieve carriers for powder types.

Friday, December 7

1) Lectures:
   a) Rhizobia - microbiology - classification and use
   b) Methods of growing rhizobia in mass
2) Exercise 4: Inoculate the glass fermentors.
3) Exercise 5: Sample the fermentors periodically for cell count.
4) Exercise 7:
   a) Determine the moisture content of the processed carrier.
   b) Determine pH of carriers and neutralize.
   c) Determine the moisture capacity ofthe carriers. Store the bulk of the carriers. These materials are to be used for inoculant production based on nonsterile carriers.
   d) Package sterile carriers in polyethylene bags for sterilization by irradiation.
   e) Package carriers in polypropylene bags for sterilization by autoclaving.

Monday, December 10

1) Lectures:
   a) Inoculant production - forms and production processes (non-sterile)
   b) Inoculant production with pre-sterilized carriers.
2) Exercise 1:
   a) Restreak reconstituted cultures from ampoules on YMA + BTB, YMA + Congo Red, and peptone glucose agar.
   b) Observe colony morphology and growth reactions on the indicatior media.

3) Exercise 2: Observe growth reactions on the media of different carbon and nitrogen sources - compare growth.
4) Exercise 4: Inoculate the glass fermentors and small steel fermentor with a fast-growing Rhizobium strain (TAL 1145).
5) Exercise 5:
   a) Test fermentor cultures for contamination by simple stain and by Gram stain.
   b) Take samples for final enumeration of cells. Make an estimation of cell numbers by comparison with McFarland standard. Streak out fermentor cultures on test media.
   c) Verify strain identity by agglutination.
   d) Review and complete records.
6) Exercise 7:
   a) Continue packaging of carriers in polyethylene bags for sterilization by irradiation.
   b) Continue packaging of carriers in polypropylene bags for sterilization by autoclaving.

Tuesday, December 11

1) Lectures:
   a) Growth and survival of rhizobia in peat.
   b) Controlling quality in inoculant production.
2) Exercise 4:
   a) Take broth samples for estimatin of cell numbers (McFarland Standard).
   b) Test for contamination by Gram stain and simple stain.
   c) Inoculate glass fermentors and small steel fermentor with TAL 1145.
3) Exercise 8:
   a) Mix powdered, non-sterile carriers withthe proper amounts of broth culture:
      1) in coffee cans.
      2) by spraying broth culture onto the carriers while agitating with the horizontal mixer. Collect in curing trays.
   b) Make inoculants by mixing broth culture with granular peat in the horizontal mixer. Collect in curing trays.
   c) Measure moisture content of all batches of inoculant.
4) Exercise 6: Calibrate Pasteur pipettes.

Wednesday, December 12

1) Lecture: Quality control of legume inoculants.
2) Exercise 2: Observe growth reaction on each medium. Record results.
3) Exercise 4:
   a) Take broth samples, estimate growth.
   b) Test for contamination (Gram stain).
   c) Perform agglutination test.
4) Exercise 5:
   a) Prepare steel fermentors for operation.
   b) Fill fermentors with water and test for proper function.
   c) Prepare media, fill fermentors, sterilize.
5) Exercise 8:
   a) Determine moisture loss in inoculants and check for pH changes.
   b) Assign lot numbers, package inoculants and store.
   c) Surface sterilize and pre-germinate soybean seed on water agar.
6) Seminar

Thursday, December 13

1) Lectures:
   a) Alternate methods of inoculum production
   b) Enumeration of rhizobia
2) Exercise 4: Check broth cultured in fermentors for purity.
3) Exercise 6:
   a) Obtain broth cultures from fermentors.
   b) Determine the total count.
   c) Measure the optical density of the broth cultures.
   d) Make serial dilutions and plate by te pour-plate, spread-plate, and drop-plate methods.
4) Exercise 4 continued:
   a) Assemble small and large glass fermentor units.
   b) Fill fermentors with liquid media, pretest and sterilize.
5) Exercise 6 continued: Surface sterilize and pre-germinate alfalfa seeds on water agar.
6) Seminar

Friday, December 14

1) Lecture: Methods of using legume inoculants
2) Exercise 8:
   a) Set up growth pouches and growth tubes.
   b) Plant seeds.
   c) Make serial dilutions of the inoculants.
   d) Perform spread plate counts of the inoculants on Brilliant Green and Congo Red.
   e) Inoculate seeds for the plant infection count.
3) Exercise 4: Inoculate glass fermentors.
4) Exercise 5: Inoculate steel fermentors (if time permits).
5) Seminar

Monday, December 17

1) Lectures:
   a) Glasshouse and growth chamber methods of evaluating rhizobia.
   b) Field testing of legume inoculants.
2) Seminar
3) Exercise 8:
   a) Make serial dilutions of inoculants.
   b) Perform plate counts of the inoculants on YMA plates containing Brilliant Green and on YMA plates containing Congo Red.
   c) Inoculate seeds in growth tubes for the plant infection counts.
4) Exercise 5:
   a) Prepare fermentors 1 and 2 for operation. Build a brick stand for fermentor 3 and prepare for operation. Make arrangements for heating with charcoal.
   b) Prepare media, fill fermentors and sterilize.
5) Exercise 10: Prepare adhesive solutions.

Tuesday, December 18

1) Lectures:
   a) Inoculant production with pre-sterilized carriers.
   b) Legume nodultion as influenced by host, environment, and effectiveness of association.
2) Seminar

3) Exercise 6: Read and calculate the viable counts obtained by the three plating methods.
4) Exercise 10:
   a) Continue preparing adhesive solutions.
   b) Weigh out six aliquots of soybean seed (200 g each) and place in jars or clear polyethylene bags.
   c) Prepare inoculant slurries for treatments as required and label -- inoculant use rate is 4.4g/kg seed.
   d) Inoculate the seeds; make a dilution series.
   e) Plate dilutions immediately after seed coating on YMA + Brilliant green.

Wednesday, December 19

1) Lecture:
   a) Compatability of rhizobia with chemical pesticides and fertilizers.
   b) Application of serological methods to inoculum production
2) Seminar
3) Exercise 8: Read and record results of the plant infection counts.
4) Exercise 9:
   a) Perform viable counts on undiluted broth cultures.
   b) Prepare 100-fold diluted culture in the dilution apparatus.
   c) Inject diluted and undiluted cultures into pre-sterilized peat in packages.
   d) Mix diluted cultures with peat autoclaved in trays and package inoculants.

Thursday, December 20

1) Lectures:
   a) Rhizobium banks: building and maintaining Rhizobium cultures for legume inoculant production.
   b) Factors affecting the response to inoculation.
2) Seminar
3) Exercise 8: Examine plates, count colonies.
4) Exercise 11:
   a) Discuss methods of soil inoculation.
   b) Prepare seedbeds.
   c) Prepare and weigh inoculants.
   d) Apply inoculants to each furrow.
   e) Add seeds.
   f) Discuss field emergency methods.
5) Awards dinner

Friday, December 21

1) Lectures:
   a) Location and management of a legume inoculation production plant.
   b) Location and management of a legume production plant.
2) Seminar
3) Exercise 10:
   a) Count colonies on YMA plates
   b) Lyophilizing Rhizobium cultures (demonstration).
   c) Last visit to the Haleakala station to inspect fermentor cultures.
   d) General discussion.

APPENDIX H. Inoculant Production Manual Contents

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APPENDIX I. Staff Travel

TRAVELLER	DATES OF TRAVEL	DESTINATION	PURPOSE
D. P. Beck	01/04-01/21/84	DELHI/LAHORE/ KATHMANDU	To confer w/USAID on AID's program in India, attend "N and the Environment" symposium in Lahore visit w/BNF researchers in Nepal.
D. P. Beck	02/06-02/22/84	SINGAPORE/AUSTRALIA	Visit w/Prof. Gloria Lim at Singapore National University re collaboration, visit CSIRO (Commonwealth Scientific and Industrial Research Organization.
D. P. Beck	05/22-05/30/84	MANILA/SINGAPORE	Discussions w/Dr. Erlinda Paterno, Southeast Asia delegate to BNFRC's steering commit tee, visit PCARRD to discuss several aspects of future training, stopover in Singapore to purchase supplies.
D. P. Beck	07/06-07/10/84	DHAKA	To attend annual meeting of P.I.'s involved in the countrywide BNF program at the request of Dr. M.A. Mannan, Member-Director of Soils Division, Bangladesh Agricultural Research Council.
D. P. Beck	07/19-07/30/84	JAKARTA/NAIROBI	To attend regional planning meeting (joint INTSOY-AVRDC-IRRI-IITA) for setup of soybean network, prior to attending AABNF meeting in Nairobi, Kenya and attend Africa Assn. BNF meeting.

D. P. Beck	08/12-08/24/84	BOGOR/JAKARTA	Visit country scientists at institutions and universities in Java to establish relations w/BNFRC and to become aware of country activities and priorities in BNF.
D. P. Beck	10/02-10/20/84	RANGOON/KATHMANDU	To consult on Burmese inoculant production/ distribution improvement, visit Soil Science Division to consult on inoculum production setup, and to discuss ongoing new cropping system projects w/USAID.
J. C. Burton	07/19-07/29/84	KENYA	To attend African Assn. of BNF, First Conference as invited NifTAL speaker.
R. J. Davis	02/01-04/02/84	GUAM/BANGKOK/	To confer w/U of Guam personnel re PL 406 Winged Beam Project, confer w/BNFRC/Bangkok re training course to be conducted by NifTAL, attend & present invited paper at Commission IV, ISSS meeting, do consultancy planning re inoculant production in Pakistan, visit ICRISAT in Hyderabad.
R. J. Davis	09/13-12/02/84	INDIA/THAILAND/ BURMA/INDIA/ PAKISTAN	To India as part of STI delegation, visit NifTAL's BNFRC in Thailand, consult with Burmese legume inoculation program re inoculant production and field trials, confer w/USAID and government of Sri Lanka officials workers, consult with M.P. Cooperative

			Oilseed Federation on inoculant production plant (this is an ongoing program initiated at request of INTSOY), confer with USAID/DELHI and ICAR scientists as follow-up on STI visit, confer w/USAID and PARC officials and follow up progress of consultancy in Feb./Mar '84 in Pakistan.
J. Halliday	01/29-02/18/84	INDIA/BANGKOK	To India as leader of Indo-U.S. Science and Technology Institution Program, visit USAID program/contract officers on 1984 work plan, and work on procedures at BNFRC, Bangkok.
J. Halliday	09/14-09/29/84	INDIA/BANGKOK	To attend INDO-U.S. Science and Technology Program, liaison with ICRISAT and coordinate w/BNFRC in Bangkok.
J. P. Roskoski	02/20-03/01/84	MEXICO	To visit and advise INLIT cooperators.
J. P. Roskoski	07/19-09/04/84	DOMINICAN REPUBLIC/ COLOMBIA/URUGUAY	To participate in symposium on BNF sponsored by CESDA for Caribbean researchers, Dominican Republic, to participate in project development and design w/respect to a proposal on BNF research and training on beans and tropical pasture legumes that CIAT is submitting to UNDP in Cali, Colombia and to teach in an inoculant production course for Latin America sponsored by FAO, NifTAL, MIRCEN, and UNESCO in Montevideo, Uruguary.

J. P. Roskoski	10/15-10/31/84	BOLIVIA/BRAZIL/	To confer w/INLIT co-operators in Santa Cruz, Bolivia and assess progress and problems of their BNF programs; attend XII RELAR BNF coordinational meeting and hold INLIT meeting in Campinas, Brazil and assess progress of tree research program in Chiclayo, Peru for former NifTAL trainee.
P. Singleton	02/11-02/17/84	AUSTRALIA	To present paper at the 7th Australian Legume Nodulation Conference in Sydney.
P. Singleton	09/14-10/10/84	INDIA	To meet and develop cooperative BNF programs w/Indian scientists.
C. van Kessel	03/02-03/18/84	INDONESIA	To attend FAO/IAEA research coordination meeting on multiple cropping systems.

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