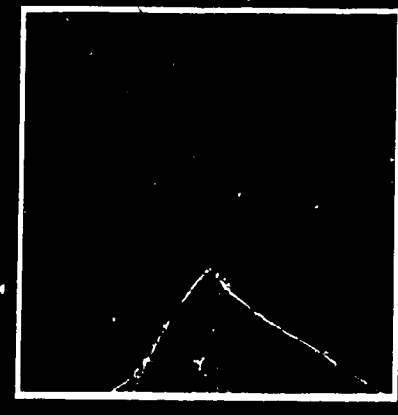
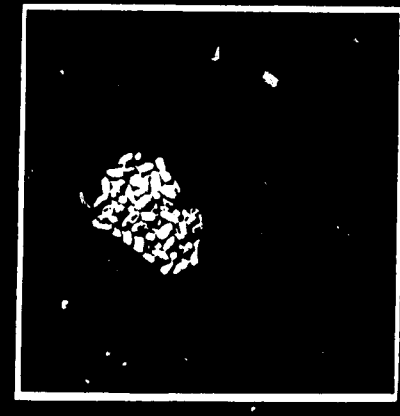
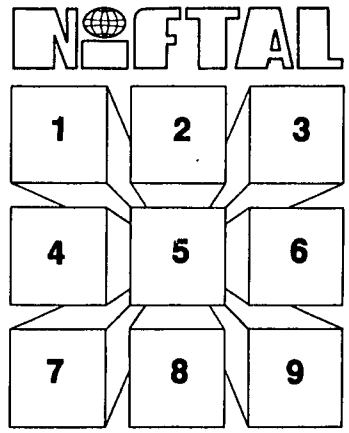
PD-ABD-892 76098

ANNUAL REPORT 1990

NOFTAL







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 per seed.

6. One of over 200 scientists participaing 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 bean (*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.

Annual Report 1990

NifTAL Project and MIRCEN

A Report for the Years 1988-1989

USAID Cooperative Agreement DAN-4177-A-00-6035-00

Dr. B. Ben Bohlool, 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

In Cooperation with

United States Agency for International Development

Statistical Summary

Title:	**Improved BNF Through Biotechnology**
Cooperative Agreement Number:	**DAN-4177-A-00-6035-00**
Principal Investigator:	**B. Ben Bohlool**
Address:	**NifTAL Project and MIRCEN 1000 Holomua Avenue Paia, Maui, HI 96779-9744 USA**
Contractor:	**University of Hawaii Department of Agronomy & Soil Science College of Tropical Agriculture and Human Resources**
Contract Period:	**10-01-86 to 09-30-91**
Reporting Period:	**01-01-87 to 12-31-89**
Total Budget:	**$4,800,000**
Obligated Funds To Date:	**$2,930,000**
Project Office:	**S&T/AGR/RNR**
Project Monitor:	**Dr. Charles Sloger S&T/AGR/RNR, SA-18 Agency for International Development U.S. International Development Cooperating Agency Washington, D.C. 20523**

Table of Contents

Statistical Summary ii

OVERVIEW

Background 5
Objectives 5
Summary of Accomplishments 6
Acknowledgements 8

OUTREACH & EDUCATION

Introduction 11

Outreach Activities

Networking and Collaboration 12
(WREN, PVO/Peace Corp/NifTAL Linkage, BNF/Legume Management)

INDO-US Science & Technology Initiative 14
BNF Resource Center 15
Collaborative Research Projects Summary 16
Material and Information Services 17
Material Services 17
(Inoculants, Antisera, Cultures)

Information Dissemination 20
Technical Assistance and Technology Transfer 21
NFTA Link 21
Technical Service Notes 22
Meeting Inoculant Demand 23
(Haiti, Uganda, Zambia)

Indonesian BNF Assessment 25

Educational Activities

Workshops and Training 26
Soil Experts Workshop 26
Science & Technology Initiative Workshop 27
PCARRD Workshop for Scientists 27
BNF Workshop for PVOs and Peace Corps 27
Inoculant Training Course in Bangkok 29
Visiting Scientist Program 29
Intern Trainees 30
Post-Graduate Program 30
Visitors 32

RESEARCH & DEVELOPMENT

Introduction 35

Environmental Constraints to Enhanced BNF 36
Rhizobial Ecology in Pastures 37
Persistence and Dispersal-Introduced Rhizobia 38
Modelling Rhizobial Survival 39
Phosphorus, Nitrogen and Temperature Effects 40

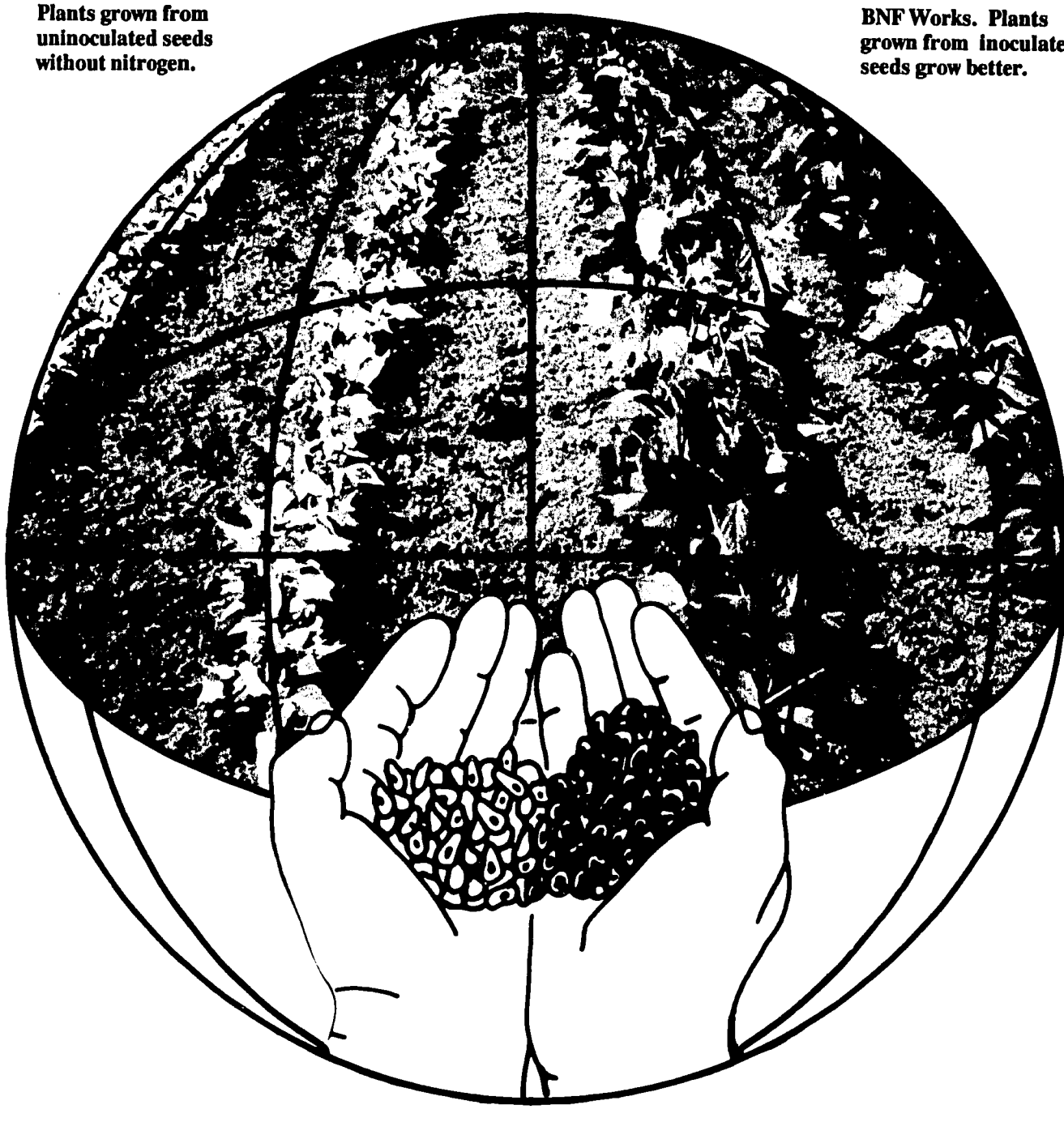
Biological Constraints to Maximal Benefit from Legume Symbiosis 42
Symbiotic Genes 42
Rhizobial Requirements of *Gliricidia sepium* 43
Nitrate Assimilation-Common Bean & Soybean 44
Inoculation Response Models 47

Methodological Constraints to Better Understanding of Legume/Rhizobia Association 48
Serological Systems 49
Single vs. Multistrain Inoculants 52
MPNES 52
Vegetable Oils as Adhesive for Peat 53

Institutional Cooperation for Solving Problems 54
India 55
Ghana 55
Korea 57
Hawaii (IBSNAT) 58
Philippines 59

APPENDICES

Grants Received from Outside Core Project 62
Abstracts of papers published in 1988 64
NifTAL Publications List 1976-1989 68
Project Personnel 75
International Staff Travel 76



In most developing countries, the small farmer's choice is not between nitrogen fertilizer or biological nitrogen fixation (BNF), but between nothing at all or cost-effective alternatives. Regardless of the price of nitrogen fertilizer on the international market, availability of fertilizer and the hard currency to pay for it is often the limiting factor.

Overview

Biological Nitrogen-Fixing Symbioses are:

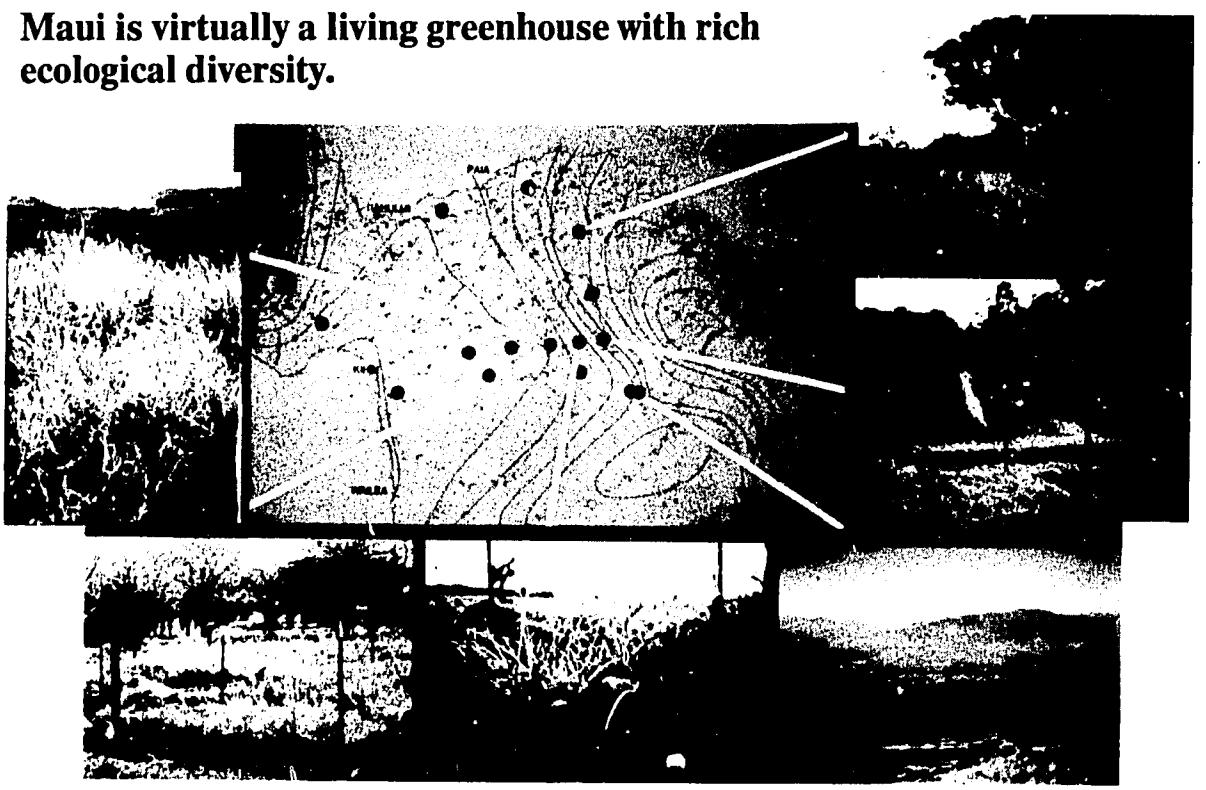
Ecologically Sound

- Mutually beneficial symbioses between plants and microorganisms are plentiful in nature and are often exploited.
- Nitrogen is the most common limiting nutrient needed to increase agricultural production in the tropics.
- Plants that fix atmospheric nitrogen can fluorish in nitrogen deficient soils without nitrogen fertilizer.
- Symbioses enhance the survival and increase the ecological range of the symbionts.
- By providing nitrogen input to natural ecosystems and agroecosystems, symbionts play an important role in nutrient cycling processes which partially determine overall ecosystem primary productivity, species composition and successional dynamics, and agricultural sustainability.
- BNF is an environmentally attractive alternative to nitrogen fertilize which is known to pollute ground and surface waters causing health and eutrophication problems

Economically Attractive

- Farmers know that rotating legume cover crops leads to dramatic yield increases of subsequent grain crops.
- Intercropping and relay cropping with legumes is the dominant form of agriculture in the world because biologically fixed nitrogen is available and cost effective for small farmers.
- Nitrogen fixed by rhizobia is cost-free if rhizobia are already in the soil.
- If rhizobia are not present, placing rhizobia in the soil along with the seed is very inexpensive. It would take at least U.S.$87 worth of urea to produce a soybean yield comparable to that possible using only U.S.$3 worth of inoculant.
- Chemical fertilizers like urea are expensive, partly because they are linked to the cost of natural gas, oil, and other non-renewable energy sources.
- Rhizobial inoculants for legumes are cost-effective and energy efficient.

Maui is virtually a living greenhouse with rich ecological diversity.



Maui serves NifTAL well as field experiments conducted throughout the island can take advantage of the ecological diversity and mimic conditions in developing countries. Our unique geography allows traveling within a day's drive to nearly all the climatic zones present in tropical countries.

Background

In 1975, at the peak of the oil crisis, the United States Agency for International Development (USAID) contracted with the University of Hawaii to implement a project to help farmers in developing countries step up agricultural production. The initial USAID contract (AID/ta-C- 1207) which ran from June 1, 1975, through June 15, 1982, drew on expertise in the College of Tropical Agriculture and Human Resources for establishment of an interdisciplinary unit called NifTAL (Nitrogen Fixation by Tropical Agricultural Legumes).

NifTAL was dedicated to the application of technologies based on biological nitrogen fixation (BNF) to international development goals. During these early years NifTAL built a resource capability in Hawaii to provide national legume programs in developing countries with research support, information, technical assistance, and multi-tiered training in BNF.

From June 16, 1982, through September 30, 1986, (USAID Contract DAN-O613-C-OO-2064-00), NifTAL continued to develop appropriate BNF technologies for tropical agriculture as well as to test these technologies in developing countries. Also during this period, technology transfer and training activities increased – particularly those related to production and use of inoculants.

Beginning in 1987, NifTAL began its second decade under a Cooperative Agreement (DAN-4177-A-00-6035-00) known as Improved BNF Through Biotechnology. This award was given based upon the continuing and expanding demand for NifTAL's services and the need to continue improving the efficiency of the symbiotic nitrogen fixing systems.

The five programs under this new agreement are: 1) Genetic Technologies for Improvement of Rhizobium/Legume Symbiosis for Crops and Trees; 2) Development of Methods for Monitoring Microorganisms; 3) Environmental Data Base to Maximize Performance of BNF; 4) Regional Resource Centers; and 5) Commercial Inoculant Technical Assistance. These programs are carried out by fulfilling the following objectives.

Objectives

The overall goal of the NifTAL Project is to reduce the dependence of developing country farmers on nitrogen fertilizer for the production of increased quantities of protein rich food. NifTAL's approach is to accomplish this goal through research and assistance to legume programs throughout the tropics to more effectively exploit the legume-rhizobia symbiosis.

Specific Objectives were:

- to determine the effect of soil infertility on symbiotic performance of legumes.
- to provide economic information on the cost of BNF technology relative to that of nitrogen fertilizers.
- to provide regional and in-county support to legume programs in developing countries.
- to train professional researchers, technicians, and extension workers in the development and implementation of legume-based BNF technologies.
- to amass and maintain a rhizobial germplasm resource comprised of rhizobial strains which effectively nodulate agriculturally important legumes under the conditions prevailing in developing countries.
- to establish an antisera bank for identifying *Rhizobium* so that the success of inoculation of legumes could be assessed.
- to develop systems for improved inoculant delivery and for insuring dependable, effective nodulation in the field.
- to identify management techniques for increasing nitrogen fixation in the field.
- to assist Private Voluntary Organization (PVO) and Peace Corp personnels in applying BNF and inoculation technology at the village level.

Summary of Accomplishments

This reporting period was a milestone in NifTAL's history as critical steps were taken toward more effective technology transfer. Predictive models of legume inoculation response were perfected and stronger links forged with a variety of development organizations and workers in Private Voluntary Organizations (PVO's) and the Peace Corps through research projects, networking, and technical assistance. Highlights of this effort included:

- Worldwide Rhizobial Ecology Network (WREN)

The international networking begun in the early 1980s (International Network of Legume Inoculation Trials) was further expanded into WREN. This work was funded jointly by the National Science Foundation (NSF) and USAID. Collaborative research was done with scientists in 22 developing countries who helped test ecological factors affecting nitrogen fixation potential. The wide divesity of tropical climates available on the Island of Maui made possible matching results with collaborative research sites overseas. The goal of this work is to build computer models which can help predict the response of legumes to rhizobial inoculation and thus make possible greater yields of high protein foods and fuelwood for developing country persons.

- Training For International Development Workers

Beginning with a mini-workshop in Washington, D.C., NifTAL launched into a major effort in collaboration with several PVOs and the Peace Corps. A two-week training workshop in biological nitrogen fixation, was aimed toward using this vital link in the effort to transfer BNF technology to developing country farmers. The result of the workshops was formation of a consortium that will lead to the solution of one of the most vexing problems faced by NifTAL staff in the past—how to get the technology directly to the farmers.

- Soil Organic Matter Dynamics In Tropical Ecosystems Workshop

Another unique event in NifTAL's history, as well as in the tropical soil field, was a workshop planned specifically to write a book on tropical soil organic matter. The workshop, funded by NSF/Ecology was a huge success as experts and reviewers met and virtually completed the first draft of the book before the week was over. The resulting book, *Dynamics of Soil Organic Matter in Tropical Ecosystems,* was released in November, 1989.

Throughout the time demands of these challenging events, research and outreach services continued by the research and support staffs. Research resulted in some exciting and useful findings and various outreach efforts touched more people than ever before. Highlights of outreach and research activities include:

- Technical Assistance

Through technical assistance efforts, inoculum is being supplied to NFTA (Nitrogen Fixing Tree Association) for use in their Cooperative Planting Program through which 3100 seed packets per year are distributed worldwide. Tree legumes were the subject of discussions with the F/FRED (Forestry/Fuelwood Research and Development Project). These discussions led to establishment of an updated list of 18 priority tree legumes. Furthermore, technical services—ranging from short visits to long term projects—were offered to Brazil, Colombia, Ecuador, Haiti, Indonesia, the Philippines, Sri Lanka, Thailand, Uganda, and Zambia.

- Visiting Scientist and Graduate Student Programs

The visiting scientist and graduate student programs were quite active with scientists from India, Korea, and Canada spending time on-site participating in collaborative research projects. Intern trainees from Burma and Korea spent several months working and learning. Five graduate students continued their studies with one person completing his Ph. D. during the year.

- Providing Materials and Information

Rhizobial cultures and inoculants, antisera, and information were distributed to more than 50 countries. A directory of BNF resource persons in developing countries was compiled to provide interested persons access, on a worldwide basis, to those with experience and knowledge about BNF. In addition, a videotape was produced highlighting the linkage between NifTAL, the Peace Corps, and PVOs.

NifTAL's outreach efforts offer our technology to those who need it through a continually expanding network of contacts and collaborators. These outreach efforts, however, are of limited value if it were not for the solid and appropriate research and development efforts carried out by the Research Section. Highlights of these activities are reported in four subsections.

- Environmental Constraints to Enhanced BNF

Using the climatic and soil diversity on the island of Maui as a natural laboratory for ecological research, the rhizobia/legume symbiosis was studied in a set of experiments to produce a model predicting both the fate and performance of introduced rhizobia. Understanding the conditions that influence the benefit farmers derive from inoculation of legume crops is imperative if valid recommendations for use and development of the technology are to be made.

Environmental factors that were studied for their influence on rhizobial colonization, nodulation, and competition were temperature, rainfall, photoperiod, and soil type. It was found that it is becoming possible to more accurately predict rhizobial behavior using the results of these studies and those of the WREN collaborators who conducted the same series of experiments in their own countries.

Assessment of the environmental impact of released rhizobia can have a far reaching effect as a model for the release of any microorganism, particularly as genetically improved organisms are developed. A two-year study following the survival of 18 strains in 18 diverse field sites yielded significant data. Populations of introduced rhizobia underwent a rapid decline upon release, followed by either survival equilibrium or complete disappearance depending on strain and/or site characteristics. The data generated by this research forms the foundation upon which models predicting the survuval of rhizobia in tropical crop systems can be constructed.

Through the studies of rhizobial survival, a computer program – MPNES – was designed to generate solutions for population estimates and the confidence intervals of most probable number data from plant infection counts. The program generates tables which help to better measure the organism of interest in microbiological studies. This software program has proven of interest to both the scientific and commercial community and is currently being used by several inoculant companies in the US in their quality control tests.

- Biological Constraints to Maximal Benefit from Legume Symbiosis

Enhancing the symbiosis requires an understanding of the dynamics of soil nitrogen and other nutrients. For example, several studies examined phosphorus management in relation to BNF and nitrogen balance in legume crop systems. Infertile tropical soils are not only low in phosphorus but may adsorb added P so strongly that applied P becomes relatively unavailable to the plant. Data on soil organic matter, organic P and N from these and future experiments will provide additional insight on how management and nitrogen fixation affect the sustainability of soil fertility.

Several experiments addressed the interaction between the rhizobial strain and the legume host. These included looking into genetic factors regulating fixation. Genus-specific microbial regulation of leghemoglobin expression in root nodules of interspecific hybrids of *Phaseolus* was the basis for a study the result of which indicates that the signal to turn on key genes in the hybrid are influenced by the infecting rhizobial strain. Another experiment looked at multiple forms of glutamine synthetase in nodules of tropical legumes inoculated with *Bradyrhizobium* spp. and *Rhizobium fredii.* The results indicate that multiple forms of glutamine synthease are probably of wide spread occurrence in legumes.

Two factors were indentified that may constrain N-fixation by *Phaseolus vulgaris:* crop growth pattern and efficient nitrate assimilation. These factors were studied in several experiments. One experiment furthered understanding of the relationship between crop N requirement, N assimilation characteristics, and N_2-fixation by both common bean and soybean. Results suggest that maximizing nitrogen derived from fixation in common bean may require altering its N assimilation characteristics through plant breeding. Research into the relationship between root morphology and nitrate assimilation by *Phaseolus vulgaris* and *Glycine max* indicated that under identical conditions common bean derived more total crop N from the soil than soybean. One benefit of increasing *Phaseolus* dependence on symbiotic sources would lead to reduction of the depletion of soil nitrogen resources that can better benefit the yield of non-leguminous plants that are part of cropping systems.

The rhizobial requirements of *Gliricidia sepium* were studied. This fast growing N-fixing tree is receiving increased attention because of its potential use in reforestration and alley cropping. Highly effective strains were identified and are being used to produce peat-base inoculants for this promising crop.

Improving inoculant production includes ways of identifying rhizobial strains in order to optimize compatibility and fixation. Serological identification of rhizobial strains is an important tool to follow the fate of rhizobia both in nature and in crop systems. Serological methods suitable to assess the performance of rhizobia in developing countries is a continuing research area. One experiment that shows promise of being useful is the immunoblot method.

- Methodological Constraints to Better Understanding of Legume/Rhizobia Association

Once a rhizobial strain is judged to be effective, the next step is to determine how to use it in inoculant formulations. One study investigated the merits of single versus multistrain inoculants. Findings suggest

that certain legumes respond as well to single as to multi-strain inoculants.

Increasing the number of nodules formed by superior inoculant strains is important when legumes are planted in fields with an indigenous population of rhizobia. Applying adhesives to seed increases the amount of inoculant that adheres to seed and in an appropriate management practice that farmers can use to increase the success of their inoculants. Certain types of preferred adhesives, gum Arabic, for example, are difficult to obtain in some developing countries. A study was done to look at an alternative adhesives and determine its compatibility with rhizobia. Several types of oils were tested. Results showed that although fewer rhizobia bind to the seed with oil than with gum Arabic, rhizobia numbers were sufficient for inoculation after 9 days of storage at 34°C. These results are encouraging since oils are usually readily available to farmers and inoculant distributors.

- Institutional Cooperation for Solving Problems

Finally, keeping transferability of technology in the forefront of our research effort is critical. Collaborative research is an important part of our technique to meet this need. A listing of our major collaborative research projects shows how these support our on-site research efforts.

The effect of crop history and evnironment on bradyrhizobial populations were studied in Karnataka State, India.

Production and conservation of fixed nitrogen in tropical crop systems and interaction between management and environment were the subjects of research in four different areas of the Philippines.

Temperature, photoperiod and mode of nitrogen nutrition effects on legume phenology were the subject of research done using data collected by IBSNAT.

The symbiotic and competitive properties of *Bradyrhizobium japonicum* native to Korean soils was studied along with a Korean visiting scientist at NifTAL headquarters.

The response to inoculation of soybean that nodulate with indigenous rhizobia in African soils were studied in cooperation with researchers in Ghana and Zambia.

Acknowledgements

The University of Hawaii acknowledges the continuing support of the United States Agency for International Development for NifTAL. The project has grown in size and capability over the years.

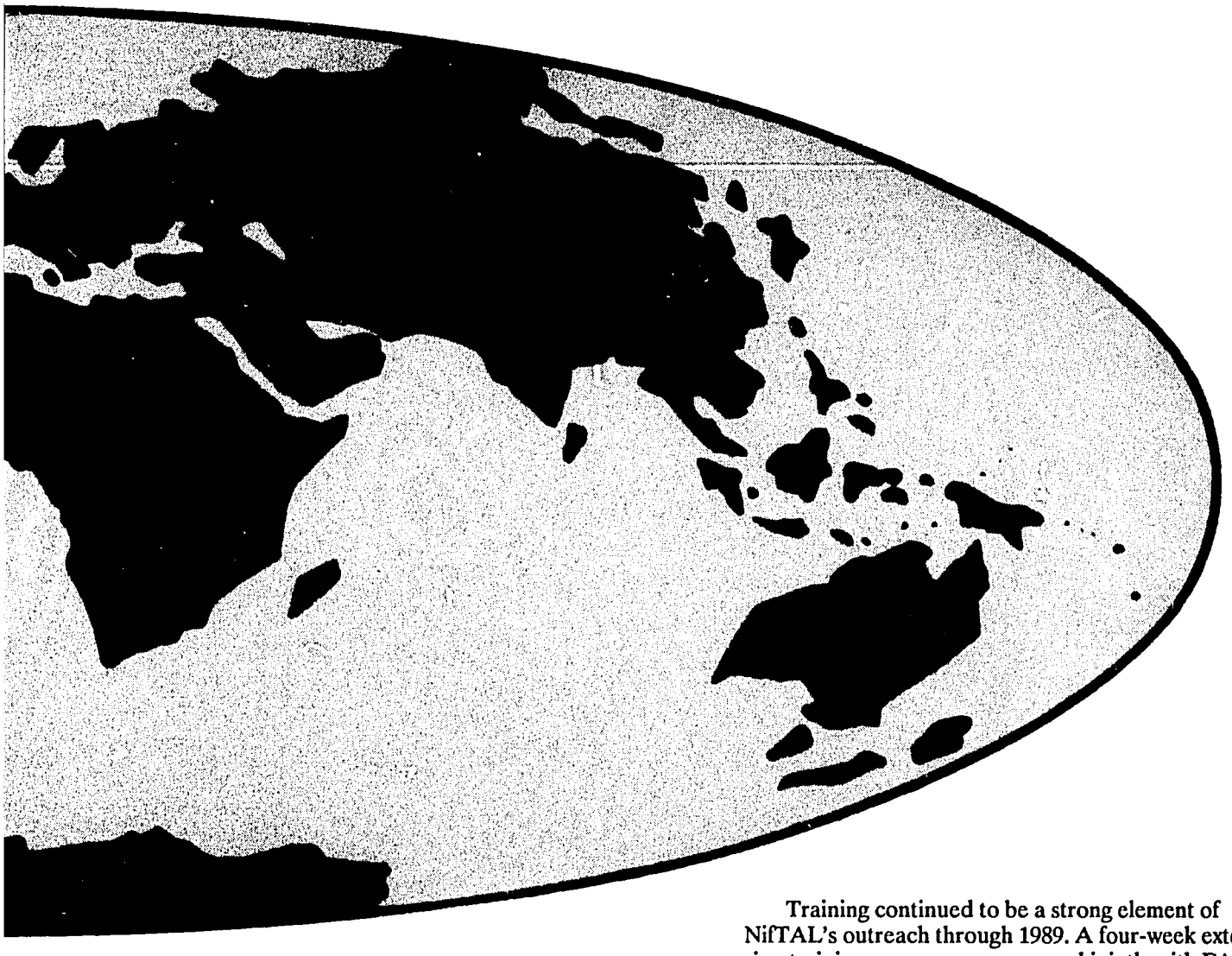
The USAID core budget for the NifTAL Project was the catalyst permitting establishment of an integrated, multi-disciplinary program augmented by many other awards from public, private, and international sources. NifTAL serves as a broad-based foundation for training and research activities funded through core operational support from the University of Hawaii and grants to individual researchers from the National Science Foundation, UNESCO, United States Department of Agriculture, the Science Advisor's Office-AID, and the McIntire-Stennis Foundation. A list of these grants is found in Appendix I.

We also recognize the support of the Mayor and County Council of Maui for provision of the 25-acre NifTAL research campus and the State of Hawaii for a $1/4 million award to the University for renovations at NifTAL over the last three years.

Outreach

&

Education



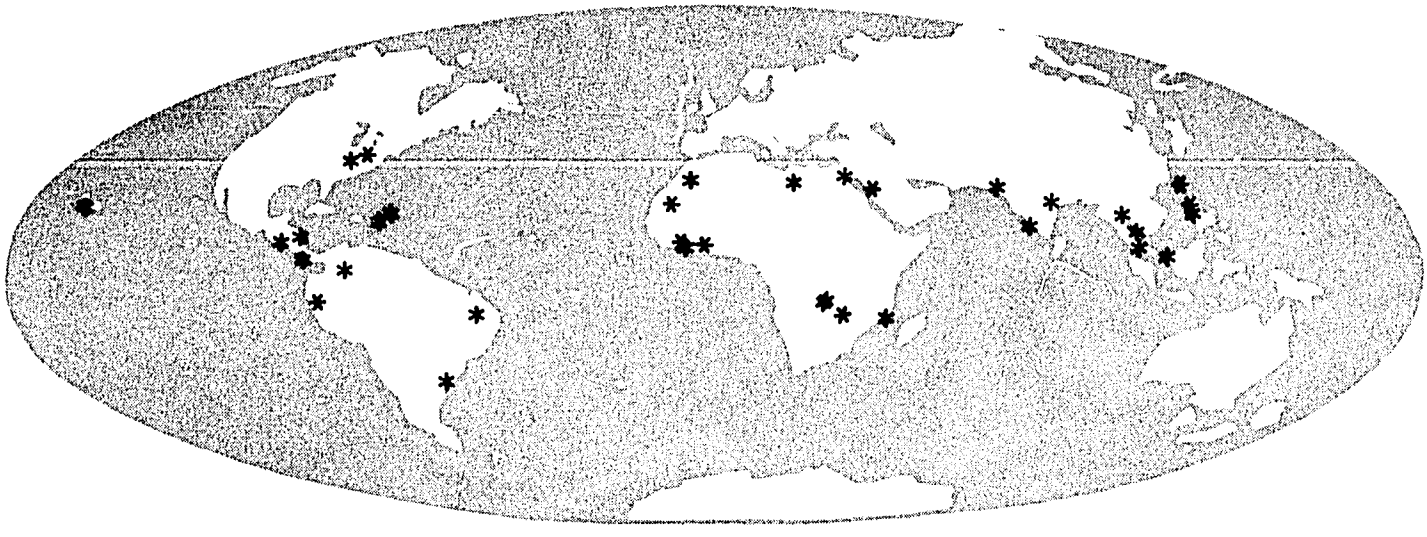
The purpose of NifTAL's outreach activities is to deliver the tools and knowledge necessary to optimize the benefits of biological nitrogen fixation in farmers' fields. This activity is directed toward developing country researchers and institutions, international development agencies, Peace Corps and private voluntary organizations. Our outreach efforts include networking and collaboration, training, workshops, technical assistance and technology transfer, and material and information services.

NifTAL networking (INLIT and WREN) and collaboration efforts reach across the globe as we move toward more predictible transferability of BNF technologies. Technical assistance and technology transfer range from responses about research techniques mailed to requestors to providing in-country BNF needs assessments and on-going programs such as those in Haiti, Thailand, and Zambia.

Training continued to be a strong element of NifTAL's outreach through 1989. A four-week extension training course was sponsored jointly with FAO and conducted at the BNF Resource Center in 1989. A two-week training workshop for PVO was held at NifTAL headquarters in 1988. In 1989, a four-week Rhizobium technology course was taught in Bangkok for scientists and technicians from South and Southeast Asia. In addition, a workshop on computer models for predicting legume response to inoculation and survival of inoculum strains in soil was held in 1989 at the University of Iowa in Ames.

Providing inoculants, cultures, and antisera allows researchers in developing countries to conduct experimental projects and enhances their inoculant production potential. Information dissemination continues to provide developing country persons with knowledge about BNF activities and current research through the continuing bibliography and computer search services, networking contacts, and training opportunities.

NETWORKING AND COLLABORATION



Reaching out in collaboration and networking (*) and through technical assistance (*).

Networking

WREN network

NifTAL is now the hub of an experimental network known as the **Worldwide Rhizobial Ecology Network** (WREN) which is partially supported by the National Science Foundation Ecology Program. Twenty-two carefully selected participants from 18 countries mostly in the tropics are collaborating with NifTAL to develop a model to predict legumes' response to inoculation which varies with legume species and site specific environmental conditions.

In order to define the parameters which affect this response, NifTAL and collaborating scientists conduct standardized pot and field trials. They also monitor the specific conditions in their diverse environments, such as the numbers of native rhizobia and soil mineral N levels, which may affect the magnitude of the response.

In 1988, the collaborators completed the characterization of their sites, conducted their greenhouse pot trials, and initiated field plantings. Data collection and analysis will be completed in 1989. The results from NifTAL and collaborating scientists will be used to construct and verify the predictive model for response to legume inoculation. A workshop of participants was held in August, 1989, to familiarize collaborators with the crop response model.

WREN International Collaborators

Mr. Robert Abaidoo (Ghana), Mr. Gustavo Bernal (Equador), Dr. Nantakorn Boonkerd (Thailand), Dr. Vernon Chinene (Zambia), Dr. Paul Davis (Zimbabwe), Sra. Consuelo Estevez (Equador), Dr. Mercedes Umali-Garcia (Philippines), Dr. S.V. Hegde (India), Dr. Ali Hilali (Morocco), Dr. Carlos Labandera (Uruguay), Dr. Kauser Malik (Pakistan), Dr. Hassan Moawad (Egypt), Mr. Ronnie Nyemba (Zambia), Mr. Michael Nyika (Zimbabwe), Dr. Erlinda Paterno (Philippines), Dr. Carlos Ramirez (Costa Rica), Mr. Walter Quadros Ribeiro (Brasil), Dr. Juan Carlos Rosas (Honduras), Dr. Eduardo Schroder (Puerto Rico), Dr. Zulkifli Shamsuddin (Malaysia), Mr. Precha Wadisirisuk (Thailand), Dr. C.C. Young (Taiwan), Dr. Fauzia Yusuf (Pakistan).

Networking for Technology Transfer Through PVOs and the Peace Corps

NifTAL took another significant step by launching a training program for private voluntary organizations (PVO) and Peace Corps (PC) workers to give them the basic knowledge necessary to get BNF technology to developing country farmers.

Cooperating in the first planning workshop, which was held in May 1988, were USAID's Office of Agriculture, Bureau for Science and Technology and Office of Private and Voluntary Cooperation, Bureau for Food for Peace and Voluntary Assistance. The second workshop, a two-week training course on BNF Technology Transfer, was held in

late 1988 for fifteen participants who shared their unique expertise in this interactive workshop.

PVO and Peace Corps participation in BNF technology transfer will provide the missing link for NifTAL toward meeting its goal of helping small farmers in developing countries produce more protein rich food and fuelwood. Combining state-of-the-art research knowledge and grassroots technology transfer techniques will result in a dynamic outreach effort.

BNF/LM Established to Reach Farmers in the Tropics

By the end of the workshop, a consensus was reached that some aspects of BNF technology are presently ready for use by the small holder farmer in developing countries. To effect use of the technology, the participants proposed that a consortium be formed between several PVO's, the Peace Corps, and NifTAL.

The consortium's purpose will be to implement BNF assessment, training, and inoculant use in developing countries. NifTAL's strength in rhizobial germplasm, research, and technology development and transfer complements PVO and PC strength in training, extension, and credit systems that exist at the farmer level in many countries.

Assessments would be made of potential benefits from improved BNF, training courses given to PVO/PC trainers on inoculant use, BNF improvement techniques, and descriptive literature (i.e., manuals and guides) produced in local languages. Additionally, inoculant production capabilities would be assessed, followed by development of a suitable system.

An outline of the formal structure and funding of the consortium was ironed out at a follow-up meeting held in Washington, D.C., in December between eight PVO and Peace Corps persons and NifTAL representatives. The consortium was funded in 1989 by AID/S&T under the title of Biological Nitrogen Fixation and Legume Management (BNF/LM) Outreach Project.

During its first year, the project is focusing its efforts in Uganda, Haiti, Nepal, Senegal, and Indonesia. The methods to help achieve the goals of the project can range from extension materials to applied research setting up collaboration between organizations.

From left: Eugene Chivaroli, Deputy Assistant Administrator for S&T, USAID; Ms. Phyllis Dobyns (at podium), Senior Vice President for Save-The-Children; Noel P. Kefford, Dean, CTAHR, University of Hawaii; Sarah Ford, Peace Corp; Ambassador Mark Edelman, Acting Administrator, USAID, Senator Daniel Inouye, Hawaii.

MIRCEN Network Connection Continues

Networking continues to all persons interested in rhizobia through NifTAL's designation as a Microbiological Resource Center (MIRCEN) charged with the preservation of rhizobial strains. Unesco provides seed funds for preservation of this important ecologial resource. The NifTAL germplam curator, Padma Somasegaran, participated in the MIRCEN council meeting held in conjuction with GIAM VII. This meeting, the Eighth International Conference on Global Impacts of Applied Microbiology was held at the Department of Biology, The Chinese University of Hong Kong, from July 30 - August 6, 1988. A poster presentation entitled "NifTAL: Specialists in Rhizobium Technology Training" was made at this meeting.

Collaboration

For most of its existence, NifTAL has operated in the collaborative mode for attaining its goals and objectives in training and education, publication, and research and development.

For training and education, NifTAL has joint-sponsored activities with Food and Agriculture Organization (FAO), the International Atomic Energy Agency (IAEA), the MIRCEN's, Organization for Economic and Cooperation Development (OECD), and several of the international centers.

Several of the manuals and informational pamphlets are produced in collaboration with the above organizations.

Indo-U.S. Science and Technology Initiative (STI)

NifTAL and the Indian Agricultural Research Institute and the G.K.V.K. University of Agricultural Sciences in Bangalore, India, have taken a broad approach to solving problems that limit the legume/rhizobia symbiosis under support of the Indo/US Science and Technology Initiative. Collaboration between the institutions has been on many levels—from simple exchange of inportant germplasm to consultation and hands-on research efforts. We have addressed limitations to the symbiosis from numerous directions always with the intent of revealing basic underlying principles rather than conducting site specific experiments.

Some of the work done under this cooperation is reported in the Research Section of this report.

Indo-U.S. STI collaborator S. V. Hegde with his graduate student.

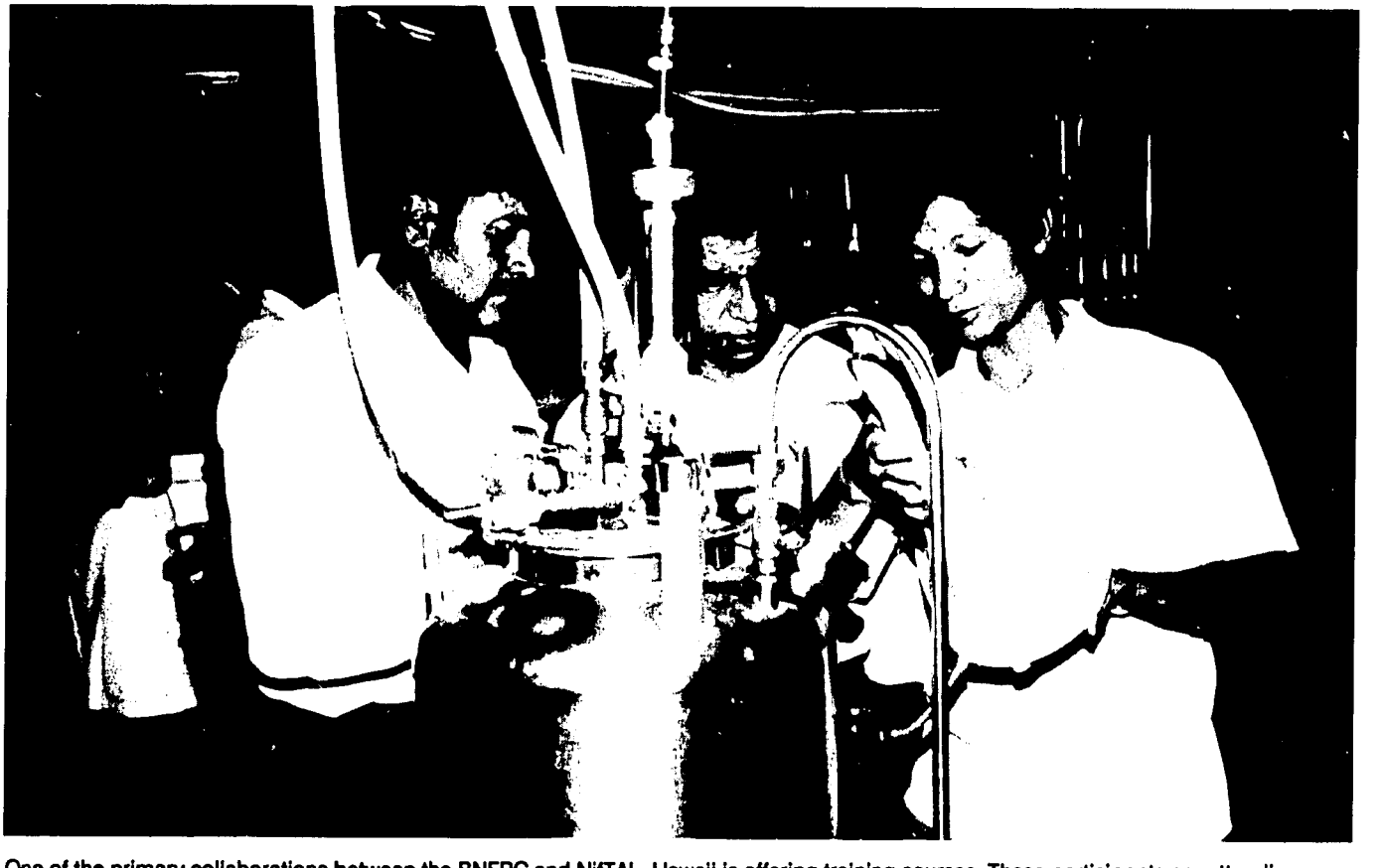
BNF Resource Center Activities in Bangkok

Because of the specialized nature of the training and research required to tailor BNF technologies to the requirements of specific locale, the Regional BNF Resource Center (for South and Southeast Asia) was established in September, 1983.

Collaborative efforts between NifTAL Project and Thailand Department of Agriculture offered the support needed in the development of practical ways to enable crops to utilize biologically fixed nitrogen (BNF). The BNFRC has encouraged research on and adaptation of tropical BNF Technology and increased food production in tropical Asia through services, training, and resource support.

Scope of Activities

- Assist national and international BNF Programs.
- Design and help implement relevant legume inoculant production and distribution system.
- Providing BNF technical assistance to country and regional pasture, oil-seed or agroforestry projects that have a legume component on a cost-basis. Collecting, testing and distributing Rhizobial cultures for research and commercial purposes.
- Promoting and facilitating the establishment of small biotechnology companies in the region by acting as an intermediary between LDC individuals and NifTAL, Hawaii.
- Hosting visiting scientists for periods of 6-12 months to conduct collaborative research with center personnel.
- Provide short-term and intern programs for LDC scientists to work with various aspects of BNF and its application in cropping system.
- Contacting USAID missions in the region to coordinate BNF technologies into USAID projects and national programs.
- Organizing in-country and regional training courses in conjunction with NifTAL Project, HI.



One of the primary collaborations between the BNFRC and NifTAL, Hawaii is offering training courses. These participants are attending an inoculant production course at BNFRC's Rhizobium Building.

Collaborative Research Projects

In order to validate and enrich its scientific program, NifTAL's scientic staff initiates collaborative research projects with international agricultural research centers, universities, and individual scientists. The listing below includes major collaborative efforts. Further details on these projects are given in the Research Section, under Institutional Cooperation for Solving Problems.

Malaysia: Evaluation of acid tolerant Leucaena and their rhizobia.

The Malaysian Agricultural Reseach and Development Institute and NifTAL Project are collaborating to select and test acid tolerant rhizobia for improved Leucaena genotypes.

Ghana: A look at soybean inoculation response with indigenous rhizobia in African soils.

The NifTAL Project and scientists at the University of Ghana are testing the response to inoculation of a soybean line that had been bred to nodulate with indigenous "cowpea" rhizobia.

IBSNAT: Testing the effects of temperature, photoperiod and mode of nitrogen nutrition.

NifTAL Project and the International Benchmark Sites Network for Agrotechnology Transfer (IBSNAT) Project are examining the interacting effects of temperature, photoperiod, and mode of N nutrition on phenology, N fixation and assimilation, and yield of soybean.

Karnataka State, India: The effect of crop history and environment on bradyrhizobial populations.

NifTAL and University of Agricultural Sciences GKVK Campus in Bangalore, India, began a three-year research program to evaluate how crop system, management, and environment affect rhizobia.

Philippines:

- Interaction between management and environment.

NifTAL and the Philippine Council for Agriculture and Resources Research and Development (PCARRD), with USAID support, developed a project that studied production and conservation of fixed N in tropical crop systems and management strategies to enhance nitrogen fixation.

- Starter N studies in Ilocos Norte

NifTAL and scientists at Marianos Marcos State University (MMU) in Batac, Ilocos Norte, carried out field experiments to evaluate the extension service's recommendation for applying starter nitrogen to legumes in the region and to determine how these recommendations affected the performance of rhizobial inoculants.

- Camerines Sur: The role of Phosphorus management in respone to legume inoculation.

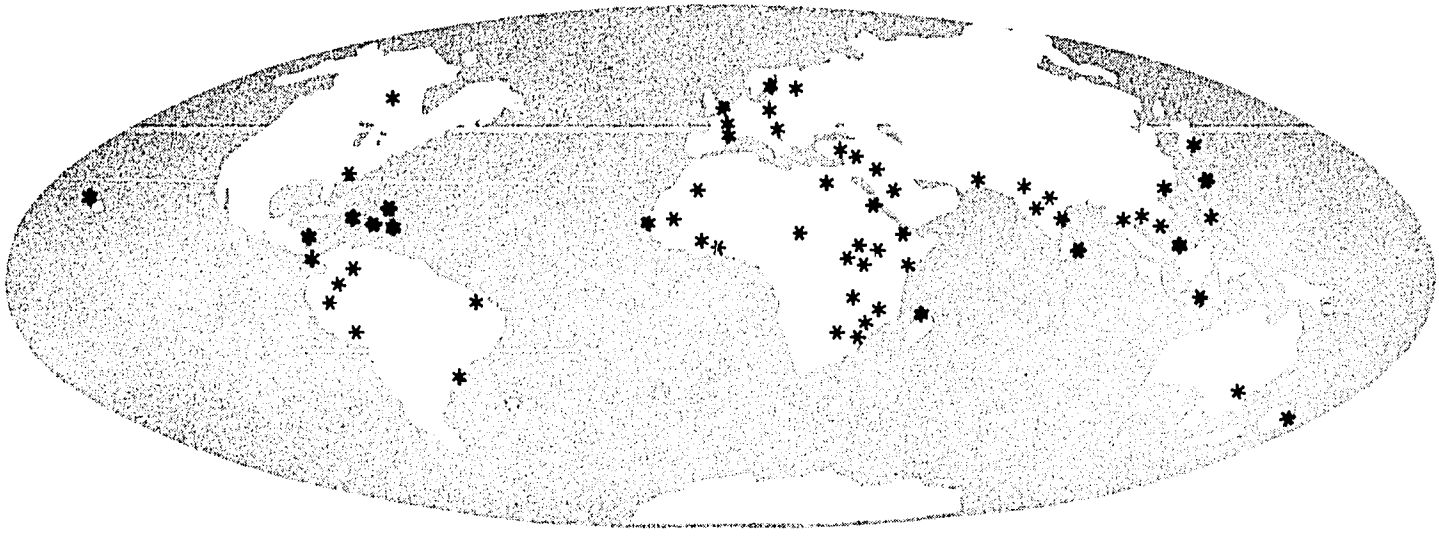
NifTAL and Camarines Sur State Agricultural College (CSSAC) collaborated to research the theory that if sufficient effective rhizobia are present either through inoculation or in a native soil population, the management inputs that increase yield will increase the need for the crop to assimilate nitrogen.

- MMSU and CSSAC: Study results aid in understanding conservation of fixed Nitrogen.

In a study of mungbean, soybean, and peanut conservation of fixed nitrogen, results indicated that P management plays an important role in N-fixation, and that the choice of species, management, and environmental interaction are critical determinants whether or not legume production will have a net benefit to soil N reserves.

Paul Singleton (center), NifTAL researcher with two of his Philippine collaborators, Heraldo Layaoen (left) and Chris Escano.

MATERIAL AND INFORMATION SERVICES



Research material distribution in 1988; additionally, information was distributed to more than 1900 persons in 100 countries.

Providing material such as inoculants, rhizobial strains, antisera, and information on research and current happenings in the BNF world are essential to meeting NifTAL's goals and objectives. Numerous requests are received each week from both the developed and developing world acknowledging the importance of this service component of NifTAL's outreach effort. This section includes several tables which summarize the distribution of materials.

Material Services

During 1988, materials were provided to requesters from more than 50 countries. Scientific support was given in the form of rhizobial inoculants, pure cultures, antisera, and seed. Inoculants were generally requested for experimental purposes; cultures for experimentation and inoculant production; antisera for identification using the fluorescent antibody technique, and seed for collaborative experiments in small research quantities only.

Inoculants

Peat-based inoculants are distributed for research purposes in connection with collaborative research in NifTAL's Worlwide Rhizobial Ecology Network. Other requests are filled for inoculants used in experiments connected with USAID, university, private voluntary organization, or national programs. In recent years, NifTAL has also provided inoculants to development projects as an interim measure prior to availability of in-country inoculant production capabilities.

NifTAL's objective in this area is to be responsive to the changing demand for inoculant types. For example, over the past several years the demand has changed from an emphasis on pasture and food legumes to tree legumes. The present concern for reforestation sparked this trend. NifTAL has responded by producing additional inoculants containing rhizobial strains compatible with a variety of popular leguminous tree species.

The following table and figure give a synopsis of types, amounts, and destination of rhizobial strains distributed.

Distribution of Inoculant Worldwide

Country	Number of Shipments/ Country	Total Kilograms/ Country	Number of Hosts*/ Country
Bujumbura	1	0.42	2
Cameroun	1	0.42	5
Chad	1	0.42	1
Costa Rica	1	0.66	4
Dominican Republic	2	1.99	6
Ecuador	3	1.00	2
Egypt	1	0.17	1
Ethiopia	1	0.09	1
Ghana	1	0.25	1
Haiti	4	80.3	41
Honduras	1	0.33	2
Jordan	1	0.25	3
Lao PDR	1	0.58	5
Lesotho	1	0.25	1
Mali	1	7.30	11
Peru	2	1.08	9
Philippines	4	3.57	22
Puerto Rico	1	0.42	1
Rep. of Cape Verde	1	0.25	1
Senegal	2	0.42	2
Sri Lanka	1	0.25	3
Sudan	2	1.08	5
Togo	1	0.66	4
Turkey	1	0.33	2
Uganda	2	0.17	1
United Kingdom	3	3.57	3
United States	14	15.72	35
West Indies	1	1.41	10
Western Samoa	1	1.00	6
Zimbabwe	1	0.30	3
	58	124.64	193

*Hosts are not unique, the same host may be counted under different countries

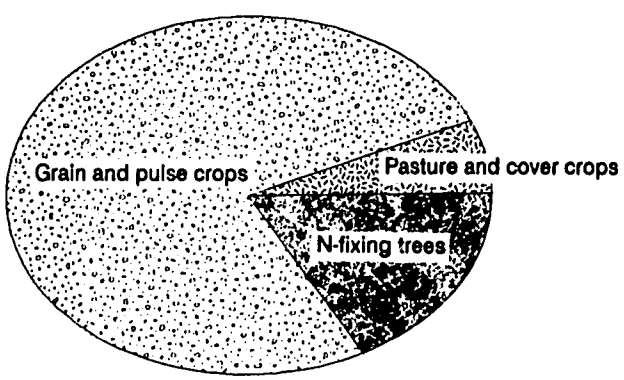
Antisera

Antisera are essential for identifying rhizobial strains recommended by NifTAL. Each strain is host specific and is serologically distinct from one another. Antisera are necessary to monitor infectiveness by the inoculant strains and for determining the long-term persistence at the experimental site. NifTAL's antiserum bank receives requests by researchers throughout the world. The table below is a synopsis of requests received in 1988.

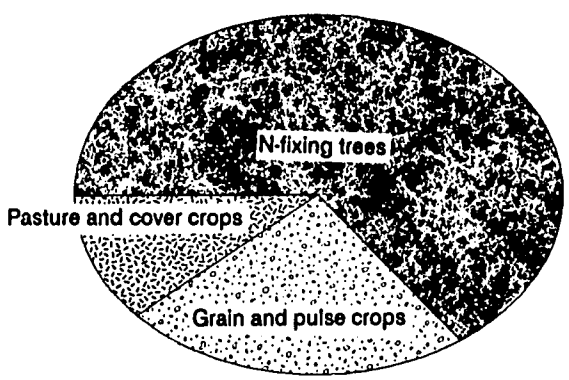
Antisera Requests Filled

Country	Number of Requests	Volume Sent
Belguim	1	45 ml
Costa Rica	1	7 ml
India	2	119 ml
Sri Lanka	2	124 ml
Thailand	2	40 ml
USA	2	47 ml
Zimbabwe	1	85 ml

Meeting the changing needs of international development programs



Inoculants types requested in the early 1980's



Inoculants types requested in the later 1980's

Cultures

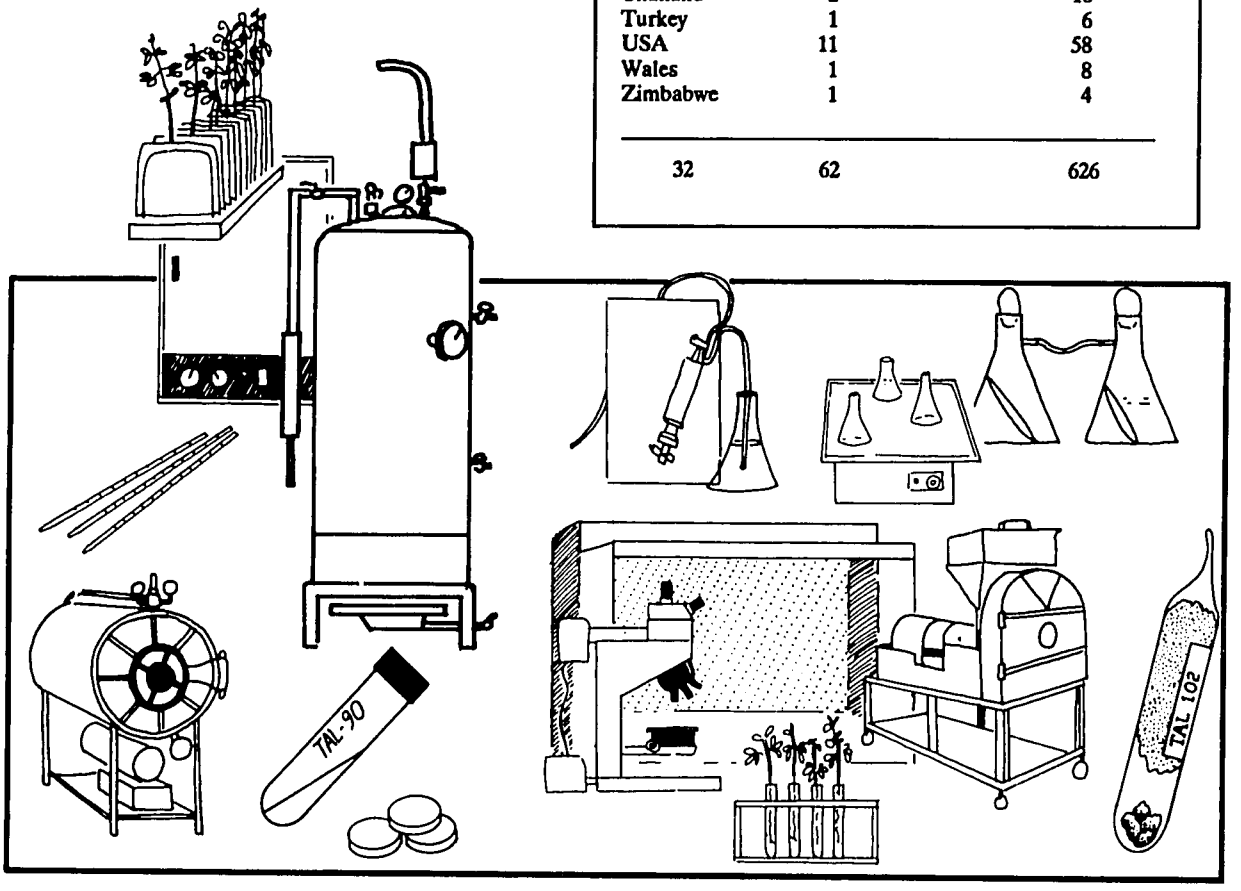
Provison of rhizobial cultures for research purposes is an important function of the NifTAL Project. Cultures preserved in the NifTAL MIRCEN collection number more than 1700. These cultures, gathered from a wide array of host plants and from diverse geographical locations, have been authenticated and tested for nodulation potential. Legume noculants are made from the strains determined to be most effective for each of the 17 most economically important legumes and for the priority tree list shown in the previous section of this report.

Over the past three years, the entire collection has been retested and preserved in ampoules as lyophilized (freeze-dried) cultures. These cultures have proven to be virtually impossible to contaminate and are superior for shipping to locations in the tropics where high temperatures cause low rhizobial populations in cultures shipped on agar slants or beads. The entire collection has also been transferred to the Indian Agricultural Research Institute in New Delhi for use and distribution in India.

The following table gives a synopsis of rhizobial culture distribution during this period.

NifTAL MIRCEN Rhizobial Germplasm Resource Services

Country	Requests	# of Cultures
Australia	1	2
Austria	1	5
Belgium	1	9
Burma	1	43
Burundi	1	11
Canada	2	16
Colombia	2	11
Finland	1	5
France	1	3
Germany	1	3
Ghana	2	15
India	12	151
Indonesia	1	4
Iraq	1	6
Japan	1	61
Kenya	1	25
Korea	1	26
Malawi	1	25
Nepal	1	14
New Zealand	1	12
Nigeria	1	9
Pakistan	1	7
Philippines	3	20
Sri Lanka	3	24
Sudan	2	21
Sweden	1	3
Syria	1	3
Thailand	2	16
Turkey	1	6
USA	11	58
Wales	1	8
Zimbabwe	1	4
32	62	626



The production of inoculants requires a number of specialized products and equipment. NifTAL has planned and makes available an inoculant production package featuring the NifTAL-designed fermenter.

Information Dissemination

NifTAL's Communication Section is charged with the responsibility of meeting requests for general information about the project and information on specific research topics This is done by filling requests from NifTAL's wide variety of publications and through providing database searches on the project's 10,000-document collection of reprints.

Following completion of the computer database in early 1988, we gained the capability of providing database searches to developing country researchers. The collection is continuously updated with current articles. As an insert to the BNF BULLETIN newsletter, our Continuing Bibliography remains a popular source of citations for current journal articles made available to requesters.

Many requests are also received for the books "Methods in Legume Rhizobium Technology" and "Legume Inoculants and Their Use." The former is a comprehensive manual teaching theory and skills necessary to work in all phases of rhizobiology. The latter is an excellent handbook especially useful to extension workers and those who need a concise overview of biological nitrogen fixation applications in the field.

Journal articles and book chapters by NifTAL staff continue to be in demand. As well, the BNF BULLETIN is sent to over 1700 interested persons in 100 countries. Also popular is the recently initiated series "Illustrated Concepts in Agricultural Biotechnology."

BNF BULLETIN Newsletter

The BNF BULLETIN gives information about the many aspects of BNF work on an international scale. Readers from over 100 countries are kept informed about BNF activities, training, and recent articles written about the legume-rhizobia symbiosis. The varied audience ranges from decision makers to extension workers in developing countries.

Tropical Soil Organic Matter Book

The book "Dynamics of SOM in Tropical Ecosystems" was released in fall 1989 by the University of Hawaii Press. The book will be available from NifTAL to eligible developing country persons for a substantial discount off the $30 price. This is a state-of-the-art book on soil organic matter produced by NifTAL as the result of a meeting of top experts in the field.

Illustrated Concepts in Agricultural Biotechnology

The illustrated concept series are one-page teaching and reporting papers. In 1988, atUnesco's request, the series became a joint publication with partial financial support frm Unesco. Number 3 in the series entitled "A Meodium-Scale Fermentor for Mass Culture of Rhizobia" and "Commonly Asked Questions and Answers about BNF," (Number 4) were published during this reporting period. Future topics to be covered in the series are genetics of BNF, serological techniques for monitoring microorganisms, and commonly asked questions and answers about BNF. A list of the miscellaneous publications produced by NifTAL is presented below.

Miscellaneous Publications

1987 Annual Report

BNF BULLETIN, Volume VIII, Number 2, with Continuing Bibliography, Number 15. (8-page newsletter with a 4-page insert.)

Directory of BNF Workers in Developing Countries (A 32-page booklet listing names and addresses of persons who work with or are interested in BNF.)

NifTAL Training Portfolio (brochure)

Rhizobial Inoculant Production and Planning Services from NifTAL (8-page booklet/brochure outlining available services and introducing the NifTAL developed fermentor for inoculant production.)

Video-Tape "Applying Science And Technology To Development - Forging the Missing Link" (presenting a model of cooperation between research projects and Private Voluntary Organizations and Peace Corps workers) 18 minutes.

TECHNICAL ASSISTANCE AND TECHNOLOGY TRANSFER

Priority tree legumes

With much international activity in the area of multipurpose tree planting, it was deemed necessary to re-evaluate NifTAL's list of priority species in order to reflect current emphasis. From reviewing current literature, and from discussions with NFTA and F/FRED (Forestry/Fuelwood Research and Development Project) staff members, the following priority list was established:

Acacia mangium
Acacia auriculiformis
Gliricidia sepium
Calliandra calothyrsus
Sesbania sesban
Sesbania grandiflora
Leucaena leucocephala
Leucaena diversifolia
Albizia lebbeck
Paraserianthus falcataria
Acacia mearnsii
Cajanus cajan (perennial habit types)
Dalbergia sissoo
Flemingia macrophylla
Faidherbia albida
Acacia nilotica
Prosopis cineraria
Prosopis juliflora

Continued efforts in selecting rhizobia that are effective, competitive, saprophytically competent, and compatible with several host species will be directed toward the species on this list.

Peat inoculant supplied To nitrogen fixing tree association

An agreement was made between NifTAL and the Nitrogen Fixing Tree Association (NFTA) for NifTAL to supply peat based legume inoculant for NFTA's Cooperative Planting Program. In this program, approximately 3100 seed packets per year are sent throughout the world for field trials. Having the appropriate rhizobia for the specific legume trees will insure adequate nitrogen nutrition during the early growth of the trees. Small packets (16 g) of peat inocula will be provided semi-annually beginning in 1989. There are currently 50 legume species in the NFTA Cooperative Planting Program.

NFTA INOCULUM DISTRIBUTION - 1989

*Group	Legume Hosts	Inoculum Group*	Amount Packets Distributed	Kilograms per Group
A:	Acacia auriculaeformis	A	66	1.06
	Acacia mangium	B	49	0.78
	Acacia mearnsii	C	75	1.20
	Acacia decurrens	D	68	1.09
	Acacia albida	E	28	0.45
			286 Total	4.576 Total
B:	Albizia lebbek			
	Albizia saman			
	Enterolobium cyclocarpum			
	Paraserianthus spp.			
C:	Leucaena spp.			
	Calliandra calothyrsus			
	Desmanthus virgatus			
	Gliricidia sepium			
	Pithocellobium dulce			
D:	Flemingia macrophylla			
	Mimosa scabrella			
	Sesbania grandiflora			
	Sesbania sesban			
	Erythrina poeppigiana			
E:	Cajanus cajan			

Countries of Distribution	Total Inoculum Packets Shipped per Country*	Packets per Group/Country A	B	C	D	E
Africa	101	19	17	28	24	13
American Samoa	3			1	1	1
Central America	4	1	1	1	1	
China	1				1	
Europe	2	1			1	
India	3	1			2	
Indonesia	6	1	1	2	2	
Lao	8	2	2	2	2	
Madagascar	9	2	2	2	2	1
Malaysia	3		1	1	1	
Nepal	2	1				1
Philippines	54	16	12	14	9	3
Rep. of Guinea	1			1		
Solomon Islands	1				1	
South America	3	1*		1	1	
Sri Lanka	4	1	1	1	1	
Sweden	1			1		
Syria	1	1				
Thailand	7	1	2	2	2	
Togo	1			1		
Tonga	3	1		1	1	
USA	63	16	9	15	15	8
United Kingdom	5	1	1	1	1	1

Note : All packets shipped were 16 grams final weight

Technical Service Notes

Among the in-country contacts with USAID missions and country nationals were the following:

Brazil

A visit was made to one of the WREN collaborators and recommendations made on plant growth, site visits made to experimental field locations, and consultations held on the experimental design.

Colombia

Discussions at CIAT yielded several types of joint ventures which could be pursued between the centers. One possibility is agroforestry activities. Additionally, discussions were held with the WREN collaborator regarding the experimental protocol.

Ecuador

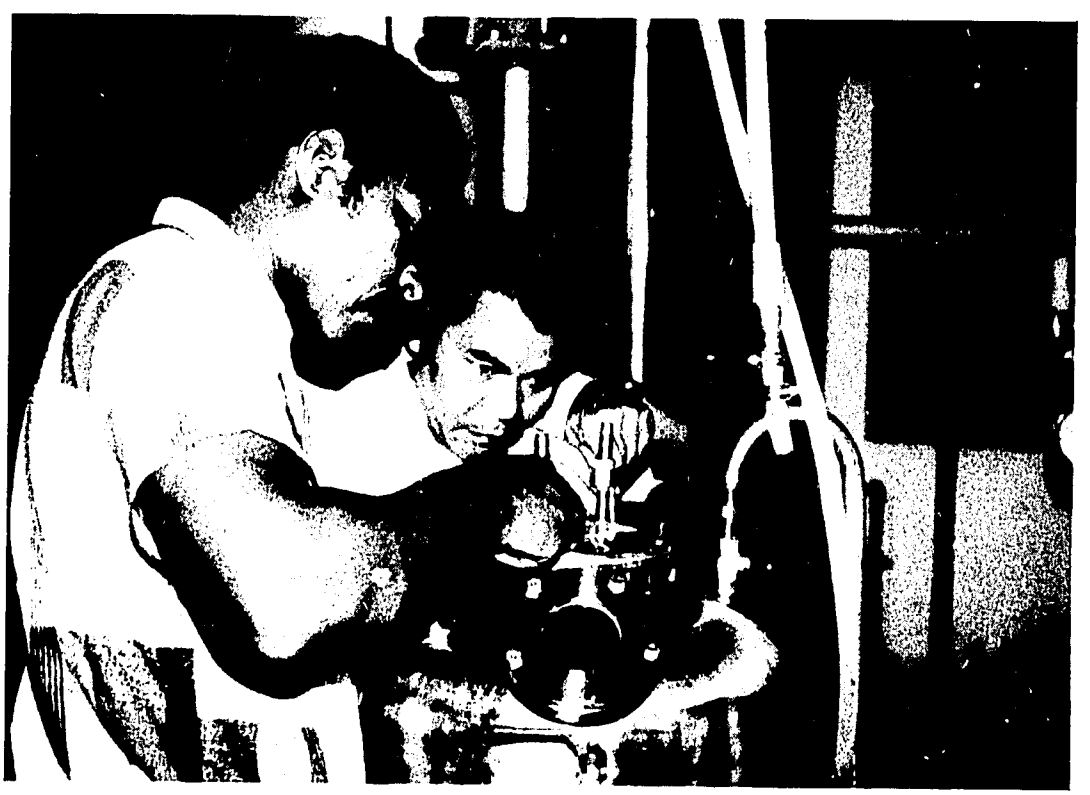
The WREN collaborator at Quito (INIAP) was assisted in selecting field sites and crops. The director of INIAP expressed interest in the idea of INIAP making inoculant for the country if money were made available for funding the venture.

Philippines

A proposal was carried out to access need for inoculation production and extension activities for AID Manila. A series of field experiments are being carried out as part of collaborative research between NifTAL and Philippine Council for Agriculture and Resources Research and Development, Camarines Sur State Agricultural College, and Marianos Marcos State University.

Sri Lanka

From April to June, Dr. Robert J. Davis provided a consultancy with the Sri Lanka Department of Agriculture's Legume Inoculation Program. The consultancy was funded by a NifTAL-USAID/Sri Lanka Buy-in. While there he worked out of the office of the USAID Diversified Agriculture Research Project in Peradeniya. The main tasks of the consultancy were evaluating the Department's soil microbiology research program, reviving and revising plans for in-country legume inoculant production, making plans for training research workers and production plant personnel at NifTAL, and for the training of extension workers in Sri Lanka. Additionally, Davis also evaluated the BNF Program of the Institute of Fundamental Studies in



Zambian inoculant producer Ronnie Nyemba and another student at the Bangkok Course assemble a NifTAL-designed rhizobial fermenter appropriate for low to medium scale inoculant production.

Kandy. Future consultancies by Davis and other NifTAL personnel were planned.

Thailand

Cooperation continues between Thai and NifTAL scientists with the BNF Resource Center as a point of contact. Bangkok is a central location for BNF activities in the Southeast Asian area.

Meeting the Increasing demand for Inoculants in Zambia

The demand for rhizobial inoculants is increasing in Zambia at a rate of 10% per year. Leadership for meeting this demand is in the hands of Mr. Ronnie Nyemba, a soil microbiologist in charge of inoculant production at Mount Makulu Research Station (Ministry of Agriculture and Water Development, P.B. 7, Chilanga, Zambia). Nyemba, received an M.S. degree in Agronomy and Soil Science at the University of Hawaii through co-sponsorship by University of Illinois' ZAMARE Project (USAID/Lusaka) and NifTAL. His training is in soil microbiology/agronomy with an emphasis on production and handling of rhizobial inoculants.

Specialized training at NifTAL and at a joint sponsored FAO/NifTAL BNF Resource Center/Thai Department of Agriculture Inoculant Production course held in Bangkok, Thailand, prepared him for his present work in his home country. Nyemba supervises inoculant production and quality control, conducts field testing of new products, trains extension agents, and is devising a marketing strategy for sale of the inoculants.

Currently, the Mount Makulu facility is producing 30,000 (150 gram each) bags of inoculant for alfalfa, leucaena, peanut, dry bean, and soybean per year. Inoculants are produced using locally mined peat from Mongu which has been processed for carrier use at Mt. Makulu. The peat is steam sterilized in autoclavable bags, then injected with a dilution of a broth culture of the appropriate rhizobia.

Quality control measures are instituted throughout the process from an initial purity check of the stock culture to a final plate count of the inoculants before they are marketed. Viable numbers of rhizobia are in excess of 1 billion cells per gram of inoculant when they leave the factory. This quality is equal to or better than commercial inoculants produced in the USA.

In farmers' fields, responses to inoculation have frequently been observed, especially on soybean. So far, all farmers requesting inoculants have been able to obtain them, but the dramatic yield response in farmers' fields has led to an increasing demand. Farmer education efforts by Nyemba through television and radio information campaigns, extension worker training, and farm demonstrations further stimulates demand for inoculants.

Mongu peat being mined for processing at Mt. Makulu. The acid peat is neutralized with lime prior to packaging and autoclaving.

To meet the rising demand, Nyemba is experimenting with producing inoculants in non-sterile peat. Early results indicate that non-sterile Mongu peat can support a large rhizobial population. The Mt. Makulu facility will also be installing two, low-cost, 100 liter stainless steel fermenters supplied by NifTAL and the ZAMARE Project. These new fermenters will increase production capacity by 200%.

Nyemba and his staff plan to take these and whatever future actions are needed to meet the rising demand for quality rhizobial inoculants in Zambian agriculture. NifTAL staff will continue to back stop these efforts as much as possible.

Minimum carbohydrate requirements for the growth of rhizobia

Among limitations to implementing successful inoculant production in some developing countries is the ready availability of certain components of the growth media and the limited amount of foreign currency available to import them. We have begun to address some of these practical concerns by examining the minimum carbohydrate requirements for adequate production of rhizobia. Initial results using inoculant-quality strains show that excellent growth rate and final yield can be achieved with one-twentieth the amount of mannitol that is normally recommended in rhizobial growth media. This was achieved with a fast- and a slow-growing strain. More detailed studies will be conducted with other inexpensive and widely used carbohydrates.

Technical Assistance in the Re-Greening of Haiti

Years of poor land use mangement, rising population, and increasing demand for agricultural and, in particular, wood products have resulted in serious environmental degradation in Haiti. NifTAL has become involved in the important effort to replace the lost forests through reforestation using fast-growing, nitrogen fixing trees. Provision by NifTAL of leguminous tree inoculants began in 1987 through cooperation with the USAID-sponsored Agroforestry Outreach Project (AOP).

Many hillside forests in Haiti have been denuded like this one.

Nitrogen fixing leguminous trees, such as *Leucaena leucocephala, L. diversifolia, Samanea saman, Albizzia lebbek, Gliricidia sepium, Calliandra calothyrsus*, and *Acacia auriculiformis*, figure prominently in the AOP program. These trees have the potential of decreasing erosion and further degredation of precious soils, as well as providing Haitians with wood products and a potential income crop.

Rhizobial inoculants have become an essential part of the nursery planting procedure. Because the tree seedlings experience severe environmental stresses upon outplanting due in part to insufficient nitrogen in the poor soils of the outplanting sites, inoculating all susceptible seedlings with rhizobial inoculants is a routine part of planting. Through 1988, inoculant continued to be supplied to key cooperators for use in tree seedling nurseries. These nurseries are sponsored by the AOP and the Pan American Development Corporation, a Private Voluntary Organization which also has a strong program in Haiti.

In 1989, NifTAL will provide inoculant production training for a Haitian at NifTAL Headquarters on Maui as well as outposting a NifTAL technician to set up a production plant in Haiti. The goal is to enable a small business enterprise to begin producing inoculants in-country in sufficient amounts to meet the critical need for legume inoculants for trees appropriate for reforestation.

Agroforestry projects can help create a continuing supply of fuelwood and a renewable income source for Haitians.

Developing a National Legume Inoculation Program for Uganda

Through support by the Manpower for Agriculture Development Project (MFAD: Ohio State University-AID-Government of Uganda), NifTAL will develop and deliver legume inoculation technology to ensure that national programs to increase legume production are not limited by insufficient biological nitrogen fixation. Over a four-year period, the activities will include training, facilities development, and an applied research program.

During a visit to Uganda, NifTAL scientist Paul Singleton initiated the activity by performing an assessment of the need for BNF technology and the level of resources available to deliver the technology to farmers. Subsequently, in November, 1989, he offered a workshop for interested individuals from industry, research, private voluntary organizations, and government planning agencies to increase awareness of the benefits of BNF technology. Currently, through support by Ohio State University and Makere University, NifTAL is helping to develop a pilot scale inoculant production facility. This activity is related to the new PVO/Peace Corp/NifTAL Consortium (BNF/LM).

Indonesian BNF Assessment

Paul Singleton recently conducted an assessment of BNF technology in Indonesia for the Government of Indonesia, USAID, and the Secondary Food Crops Development/Communication for Technology Transfer in Agriculture Projects. The assessment developed a projection of the expected benefit to farmers from inoculating their crops. In this context, constraints to delivery of the technology were evaluated. It was found that the potential supply of high quality inoculant was in excess of demand and that institutional capability to conduct research is adequate.

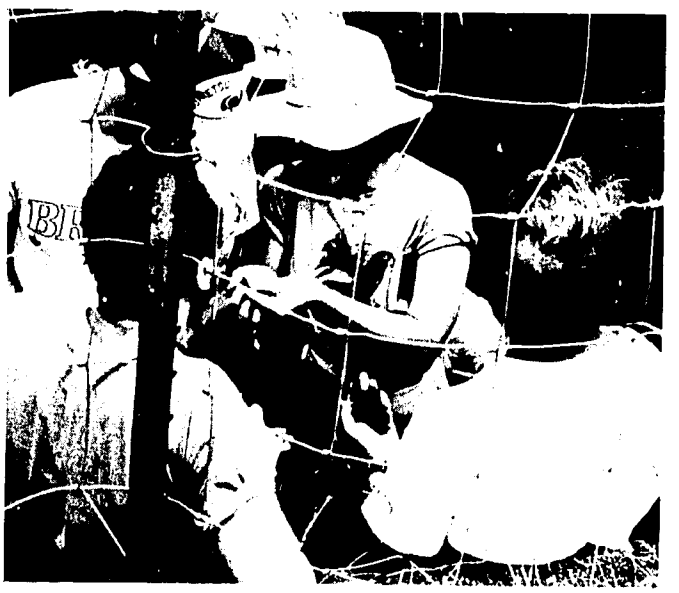
The relatively small use of inoculant by farmers was associated with the lack of understanding of the use and economic benefits of this technology by extension personnel and farmers. To address this weak link to further implementation of BNF technology in Indonesia, NifTAL proposed conducting a BNF Technology Training Course for Indonesian extension workers who are active in farmer training. The course will be held in Thailand in conjunction with NifTAL's BNF Resource Center (Bangkok). This proposal is under consideration by the Communication for Technology, Transfer in Agriculture/Secondary Food Crops Development Products (AID-Indonesia and the Government of Indonesia).

Training Program and Workshops

Workshops

Soil Organic Matter Dynamics Experts Gather

From October 7 to 15, 1988, NifTAL hosted a workshop which brought together a group of expert scientists for a unique purpose. The primary task of the event was to produce a book on soil organic matter (SOM) dynamics. This workshop was funded by a grant from the National Science Foundation's Ecology Program. Meeting participants worked under the general agreement that organic matter can play an important function in alleviating nutrient and stress problems in soils. But, very little is known about the specific biological processes involved in transformation of soil organic matter (SOM) and organic matter inputs into ecosystems. This is particularly true of tropical soils dominated by variable- charge clays and subjected to heavy rainfall over a long period of time. It is well known that highly leached tropical soils have high levels of acidity and toxic metals and low amounts of available nitrogen, phosphorus and the bases.



Soil scientists engrossed in examining soil at a high elevation site on Maui.

This SOM book will be the result of a process that began with a questionnaire distributed in July of 1986 and ended with a workshop in October 1988. From responses we received to these questionnaires about tropical SOM sent to over 100 scientists, seven topics were selected as major themes for this workshop and the chapters of the book.

Teams of experts were commissioned to write a "position paper" on each of the seven themes. Authors were asked to be deliberately controversial and provocative. Each "position paper" was then submitted for evaluation to a separate panel of experts from such diverse fields as ecology, chemistry, physics, soil science and agriculture.

During the week-long workshop the authors and the reviewers gathered together to defend their positions, reconcile differences, and produce "consensus papers" that have become the book's seven chapters. The book also identifies gaps in knowledge, prescribes corrective measures, and formulates research priorities related to ecological interactions that regulate organic matter dynamics in tropical ecosystems. *Dynamics of SOM in Tropical Ecosystems* is available from University of Hawaii Press. The book is available to people from developing countries at a 40% discount when ordered directly from NifTAL.

Indian and U.S. scientists gather to design experiments in the INDO-US S.T.I.

STI Workshop

The Third Joint Workshop of Indo-U.S. Science and Technology Initiative (STI), and the Senior Scientific Panel (SSP) Program in Agriculture was held in Bangalore, India, in 1988. Thirty Americans and 42 Indian scientists involved in the STI Program attended the workshop. The workshop especially provided an opportunity for NifTAL staff scientists Ben Bohlool and Paul Singleton to meet with collaborating scientists to plan for and assess progress on their research projects.

The joint workshop highlighted 1) the initial objective and relevance of Indo-U.S. scientific cooperation; 2) its progress during the first three years; 3) rationale resulting in updating the Davis Workshop; 4) the progress since then; 5) appraisal of the future technical scope up to 1991; and 6) the topics jointly recommended at the high level panel meetings. Workshop topics included nitrogen fertilizer use efficiency, molecular biology of BNF, Azolla and Blue-green Algae, and Biological Nitrogen Fixation in legume/rhizobia systems.

Rodale Workshop Presentation

A NifTAL microbiologist participated in a workshop (October 13-15, 1988) on "Methodologies for screening legume germplasm for use in soil improvement" organized by the Rodale Research Center, Pennsylvania. He made a presentation entitled "Rhizobial Requirements of Some Underexploited Legumes."

PCARRD Hosts BNF Workshop for Local and International Scientists

NifTAL's head of research, Paul Singleton, and Biological Nitrogen Fixation Resource Center (BNFRC) director, Nantakorn Boonkerd, participated in an evaluation and planning workshop in the Philippines, July 10 -11, 1989. The workshop, Biological Nitrogen Fixation (BNF) on Legumes, was held at the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD) Headquarters, Los Banos, Laguna, Philippines. Dr. Singleton presented a paper on Assessing the need for inoculants: Promising Technologies that Remove the Site Specific Nature of Inoculant Trials.

The purpose of the workshop was to assemble local and international scientists in order to assess the state-of-the-art on BNF and identify research and development activities as well as institutional and economic constraints in implementing BNF technologies at the farm level.

BNF Technology Workshop for PVOs and Peace Corps

The NifTAL Project followed up a one-day seminar in May designed to introduce BNF Biotechnology to Private Voluntary Organization (PVO) and Peace Crops workers with a 2-week technical workshop held at NifTAL's Maui headquarters from October 24 through November 4. Fifteen participants representing the Peace Corps and PVOs-Africare, Agriculture Cooperative Development International, CARE, Catholic Relief Services, Opportunities Industrialization Center International, Inc, Pan American Development Corporation, Save The Children, and World Vision. They shared their unique expertise in this interactive training.

This workshop, designed especially for PVOs and Peace Corps, followed the BNF process from theoretical information through microbiological laboratory and field techniques to inoculant production and, finally, to potential application of the technology in the farmer's field. Participants gained knowledge through lectures, practicums, demonstrations, field trips, and discussion groups.

About the Participants

Joseph Wilmer Alix, Project Manager, Haiti, Save The Children
Omar Jadallah, Project Manager, Jordan, Catholic Relief Service
Scott Josiah, Nursery Specialist, Haiti, Pan American Development Foundation
John Michael Kramer, Director, Agriculture and Natural Resources, CARE
Julia Morris, Associate Peace Corps Director, Mali, Peace Corps
Nathan Pagasura, Associate Regional Peace Corps Director, Region 8, Philippines, Peace Corps
John Preissing, Project Manager, Catholic Relief Services, Peru
Tanay Rema, Field Manager, World Vision Relief and Development Inc, Bangladesh
Suzanne Rucker, Director of Information, Agricultural Cooperative Development International
Gandhi Selvanathan, Agricultural Training Specialist, Opportunities Industrialization Center International, Inc.
Henry Taylor-Cline, Program Advisor, Togo, Opportunities Industrialization Centers International, Inc.
Hilberto Ugalde, Associate Peace Corps Director, Costa Rica, Peace Corps
Remko Vonk, Assistant Director, Agriculture and Natural Resources, CARE
Robert Wilson, Rural Development Specialist, Africare
James Worstell, Technical Advisor, Save the Children

Remko Vonk, Care, and Julia Morris, Peace Corps, discuss the growth pouch authentication technique.

Peace Corps and PVO development workers and NifTAL scientific staff members.

Training

The heart of NifTAL's training program is hands on experiential learning by course participants. Pictured here are Banyong Toomson (foreground) and Nora Armones practicing inoculating a laboratory scale fermentor.

Inoculant Production Training Course Held in Bangkok

The NifTAL BNF Resource Center organized a training course in inocualnt production with support from the Department of Agriculture, Thailand, FAO, and NifTAL. Sixteen participants - two each from Bangladesh, Indonesia, Lao PDR, Nepal, Philippines, Sri Lanka, Thailand and Vietnam - attended the course which was held from March 6-31, 1989.

The objective of this course, "BNF Technology and Inoculant Utilization," was to train young scientists in the member countries to be able to handle rhizobial inoculant properly, so that they can adapt the use of inoculant effectively in their countries.

Visiting Scientist Program

Visiting scientists working at NifTAL Headquarters during 1988 were Ui Ho Park (Korea), Y. D. Guar and S. V. Hegde (India), and Brian Holl (Canada). Intern trainees were Ui Gwm Kang (Korea), and Nwe Nwe Aung (Burma).

Y. D. Gaur

Dr. Y. D. Gaur, a research scientist from Indian Agricultural Research Institute, New Delhi, collaborates with NifTAL as part of the INDO/US STI Project. While at NifTAL he worked with H. Hoben on the production of antibodies against serologically recalcitrant strains of *Rhizobium* sp. of chickpea. The research was designed to provide a research tool for the identification of efficient nitrogen fixers. Good quality antisera are required to monitor these rhizobial populations and thus assess their performance under different environmental conditions.

S. V. Hegde

Dr. S. V. Hegde, Indian Agricultural Research Institute, Bangalore, is another Indian scientist collaborating in the INDO/US STI Program. During his stay he initiated studies on the survival of rhizobia in different inoculant carriers. He is also a WREN collaborator and has collaborated in other studies on rhizobial populations in different soils of India.

Eui Ho Park

Dr. Eui Ho Park, a plant breeder from Gyungnam-do, Korea, participated in research on strain-host regulation using the split-root techniques developed at NifTAL. He also worked with hybrids of the nitrogen fixing tree Leucaena. The hybrids were tested for nodulation with a number of different rhizobial strains. His work resulted in an increased knowledge about this useful fast growing tree. Dr. Park was sponsored by his government.

F. B. Holl

Dr. Holl, Associate Professor, Department of Plant Science, University of British Columbia, Vancouver, Canada, conducted research. He participated in the PVO Workshop (October-November) and presented a talk on genetic engineering for BNF to the session on specificity and promiscuity in the legume/*Rhizobium* symbiosis.

Holl's research results implied that oxygen supply and nodule O_2 tension are critical factors in the regulation of specific nodule activity. His research at NifTAL described a significant host x strain interaction for N_2 fixation activity in interspecific hybrids of *Phaseolus acutifolius* and *P. vulgaris*. These parental species both lack the common nodule leghemoglobin (Lb) heterogeneity observed in most other species. The key role of this protein in nodule oxygen metabolism in the interspecific hybrids to evaluate Lb variability in relation to the symbiotic response is an area for future research.

Intern Trainees

Ui Gwm Kang

Mr. Ui Gwm Kang from South Korea was an intern trainee from January to November 1988. He was from the Yeong Nam Crop Experiment Station, Milyang. Mr. Ui Gwm worked on the symbiotic and ecological characteristics of *Bradyrhizobium japonicum* in native Korean soils. His program was supported by the Korean government.

Nwe Nwe Aung inoculates a NifTAL-designed fermentor with rhizobial culture.

Nui Nui Aung

Ms. Nui Nui Aung was an intern trainee from January to March 1988. She is from Burma and was sponsored by the Food and Agricultural Organization of the United Nations (FAO). During her training at the NifTAL Project, Ms. Aung was involved in a research project investigating the use of vegetable and other oils as adhesives for seed inoculation using peat-based inoculants on chickpea, peanut, soybeans, and beans.

Graduate Students

One graduate student, Thomas George, completed his work at NifTAL in December. Continuing their work are Janice Thies, Dan Turk, Paul Woomer, and Maria Luz Caces.

Doctoral candidates Thies and Woomer are working on a project designed to develop a database to predict legume response to inoculation based on soil rhizobial populations and soil N availability. Caces is researching the competition of rhizobia for nodule occupancy. Dan Turk, a Master's Degree candidate, has been completing his coursework requirements on the Manoa campus.

Thomas George

Dr. George completed his Ph.D. in Agronomy in December. The title of his dissertation is "Growth and Yield Responses of *Glycine max* and *Phaseolus vulgaris* to Mode of Nitrogen Nutrition and Temperature Changes with Elevation." This research was conducted at three sites along an elevational transect within the MauiNet (Maui soil, climate, and land use network).

In mid-year, Dr. George received the Potash and Phosphate Institute's J. Fielding Reed Fellowship award for outstanding graduate research. He received a certificate and a monetary award.

A paper entitled "Yield, Soil Nutrition Uptake, and Nitrogen fixation by Soybean from Four Maturity groups grown at Three Elevations" was published in Agronomy Journal. Three other papers are in process or have been submitted. The subjects of these papers are temperature and nitrogen effects on phenology of soybean, growth responses of soybean and common bean to elevation and mode of nitrogen nutrition, and the relationship between nitrogen assimilation characteristics and nitrogen fixation in soybean and common bean. Dr. George attended the annual meeting of the American Society of Agronomy where he presented a paper on the contrasting pattern of nitrogen assimilation between soybean and common bean.

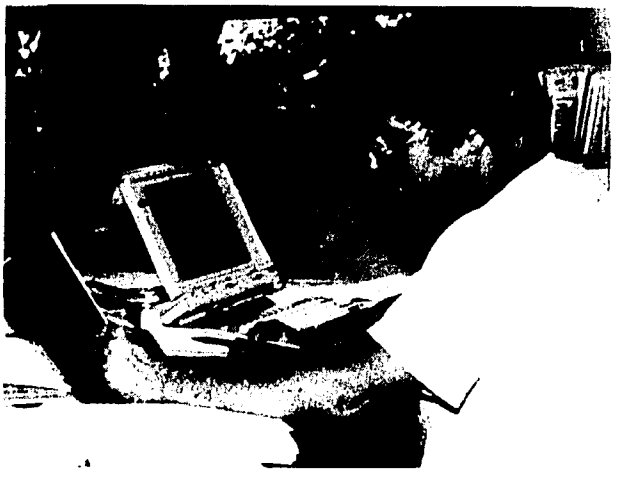
Thomas George

Paul Woomer

Paul Woomer's dissertation research concerns the development of predictive models of rhizobial survival based on measurable environmental parameters. The soil and climatic diversity of Maui is being used as a research tool through the introduction of 18 elite strains of rhizobia into 18 field sites.

The persistence of the rhizobia was monitored for two years. This persistence was compared across a comprehensive database describing the sites. Mr. Woomer will complete his studies by May 1990.

During 1988, Mr. Woomer published two articles in the Journal of Applied and Environmental Microbiology. The first, "Ecological Indicators of Native Rhizobia in Tropical Soils" (June, 1988), allows for the prediction of the population size of indigenous rhizobia in soils. The second, "Reliability of the Most-Probable-Number Technique for Enumerating Rhizobia in Tropical Soils" (July, 1988), demonstrates that plant infection counts of rhizobia may be compared across soils but not always across legume hosts.



Mr. Woomer attended three conferences in 1988. He coauthored a paper with Dr. B. Bohlool and Ms. J. Thies "The importance of soil characterization in studies of Rhizobium ecology," for the meeting of the Western Regional Cooperative Soil Survey Conference (Kahului, Maui, June 1988). He coauthored a chapter in the special ASA/CSSA/SSSA "The Persistence of Forage Legumes" with B. Bohlool; this work was presented at the Trilateral Conference on Problems with Forage Legume Pesistence (Honolulu, HI, July, 1988). At the American Society of Agronomy meetings (Anaheim, CA, November 1988), he presented two papers, "The Most-Probable-Number Enumeration System (MPNES)" and "Selecting Rhizobia for Persistence in Tropical Soils."

Mr. Woomer is the coauthor of the Most Probable Number Enumeration System, a computer software program useful in the enumeration of microorganisms in soils, foods, and water. This program was distributed during 1988 throughout the private sector, research laboratories, and regulatory agencies.

Dan Turk

New master's degree candidate, Dan Turk, joined the NifTAL Project beginning in the fall semester. He is currently attending classes at the Manoa Campus. The course work section of his program concludes in Spring 1989 after which he will be conducting research at NifTAL Headquarters on Maui. Immediately prior to coming to NifTAL, Mr. Turk was involved in agroforestry programs in Zaire. His research will address rhizobial requirements in the symbiosis of several promising multi-purpose leguminous trees.

Janice Thies

Doctoral candidate Janice Thies continues work on a project designed to develop a database to predict legume response to rhizobial inoculation. The last two of eight field experiments necessary for development of predictive models were completed in 1988. All field experimental data have been summarized and analyzed. Preliminary response models were developed using multiple regression analysis. The models incorporate measures of soil rhizobial populations and soil N availability as the primary predictors of inoculation response.

During the PVO workshop, Thies presented two lectures entitled "Basic Philosophies of Experimental Design and Sampling" and "The Importance of Native Rhizobia on Legume Performance."

She completed the first half of her teaching requirements during the fall 1988 semester by instructing the Microbiology 130 laboratory and presenting class lectures at Maui Community College.

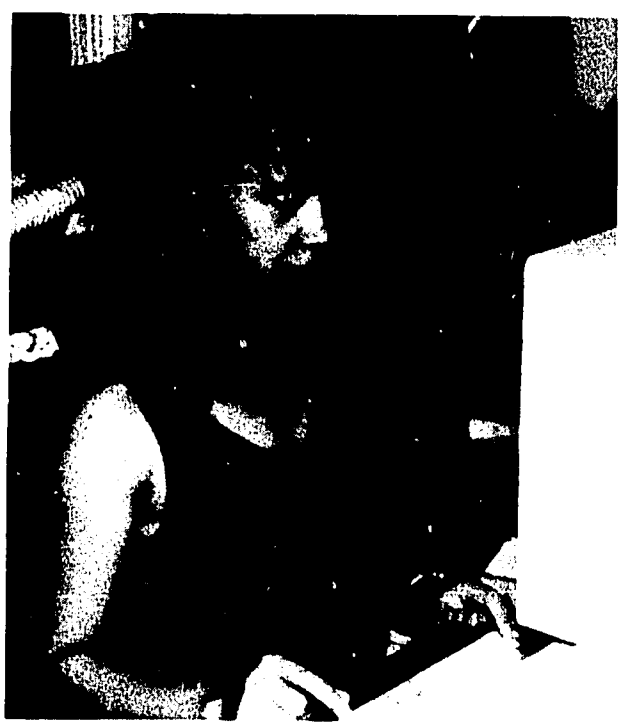
In 1988, Ms. Thies produced a special work unprecedented by any other NifTAL graduate student. This special work is her daughter known as Anneliese and she was born April 3.

Janice Thies

Maria Luz Caces

Ms. Caces dissertation research project deals with the study of competition among *Rhizobium leguminosarum* bv. *phaseoli* strains for nodule occupancy. In the first quarter, she finished characterizing six strains for their competitiveness and effectiveness on several *Phaseolus vulgaris* (common bean) cultivars. These strains were also characterized for plasmid content, intrinsic antibiotic resistance, and periplasmic protein pattern. Strain TAL 182 was selected as the most promising strain for genetic manipulation because it is highly competitive and effective.

In June, Caces traveled to Cold Spring Harbor Laboratory on partial NifTAL support to take the "Advanced Bacterial Genetics" course where she was trained in molecular biology techniques. She submitted the first draft of her research proposal on the "Isolation and Characterization of Competition Defective Mutants of *Rhizobium leguminosarum* bv. *phaseoli*." For the last quarter of the year, Caces generated a collection of TAL 182 transposon mutants for screening of their competitive ability in a plant assay.



Luz Caces

Distinguished Visitors

Among the many visitors to the NifTAL Project were the following:

David Coleman, University of Georgia, USA

Hsii Ho Ching, Taiwan Forestry Research Institute, Taiwan Dunstan Spencer, IITA, Ibadan, Nigeria

Clive Ronson, Cambridge, MD, USA

Bill Lowther, Ministry of Agriculture, New Zealand

K. S. Bhatia, N. K. Verma, M. Saleem, R. S. Dhanda, K. S. Bangarwa, and M. P. Diwakar, India

Carl-Goran Heden, MIRCEN/Stockholm

R. Senaratne, University of Ruhuna, Sri Lanka

Ed Appelbaum, Agrigenetics, USA

Saono Susono, Dept. of Agriculture, Indonesia

Perry Olsen, Canada

Ron Gollehan and Donald Crane, Agricultural Cooperatives Development International, USA

Charles Kabuga, Uganda Cooperative Alliance, Uganda

Nasir Malik, Battelle Corp., USA

U Siang Uk, U Thaung Tin, Burma

Philip Dovo, Assistant Director of Agriculture for Vananatu

T. Yokoyama, TARC, Japan

Patrick Flanagan, Central Michigan University, USA

Marianne Sarrantonio, Rodale Research Center, USA

K. A. Ronaweera, P. B. Rombukuella, B. Palarajah, B.A.S.S. Padmalal, Sri Lanka

Soroso Sindhoesarajo, SFCDP-USAID, Indonesia

Tin Hla, Tun H. Laing, Myat Twe, Burma

Dawn Koetkott, University of North Dakota, USA

Research

&

Development

30
25
10
5
CM
5
10

The nature of the legume/*Rhizobium* symbiosis provides the basis to develop a diverse and rich program of basic and applied research. There are few aspects of agriculture that are as economically important and intellectually challenging as this symbiosis. The challenge for the research program at NifTAL is to identify, among the multitude of potential research projects, problems whose resolution will be of near term benefit to farmers, extension workers, and scientists working to increase agricultural productivity in the tropics. NifTAL's mandate for research is neither commodity nor regionally oriented. As such, emphasis is placed on research problems which address general principles of the symbiosis and which have wide applicability.

Although sophisticated techniques, hypotheses, and terminology frequently characterize research at NifTAL, there is a sound justification for the projects; a justification that involves realistic consideration of problems that limit the full exploitation of the symbiosis by farmers in the tropics.

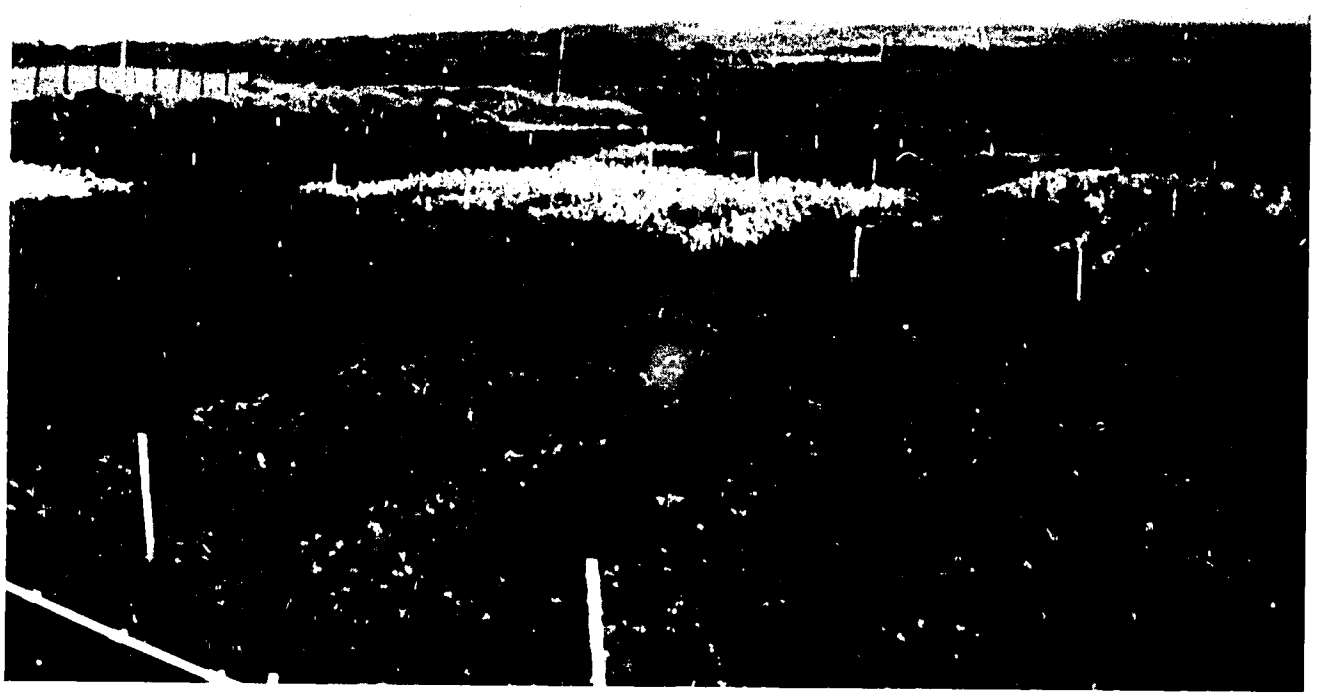
NifTAL is a small project that can address only a fraction of the research the legume/rhizobia symbiosis requires to fully understand this complex system. The Project has made an effort to identify research areas for which there is a comparative advantage. To implement the research program, the resources have been organized into three objectives concentrating on a research area: 1) environmental constraints to the performance of rhizobia; 2)host-strain interactions; and 3) inoculant technology development. This management approach has encouraged cooperation and integration of projects. The end result will be to produce more significant research results. This report includes information gained from projects in the three areas listed above, plus a 4th section on collaborative research.

Environmental Constraints to Enhanced BNF

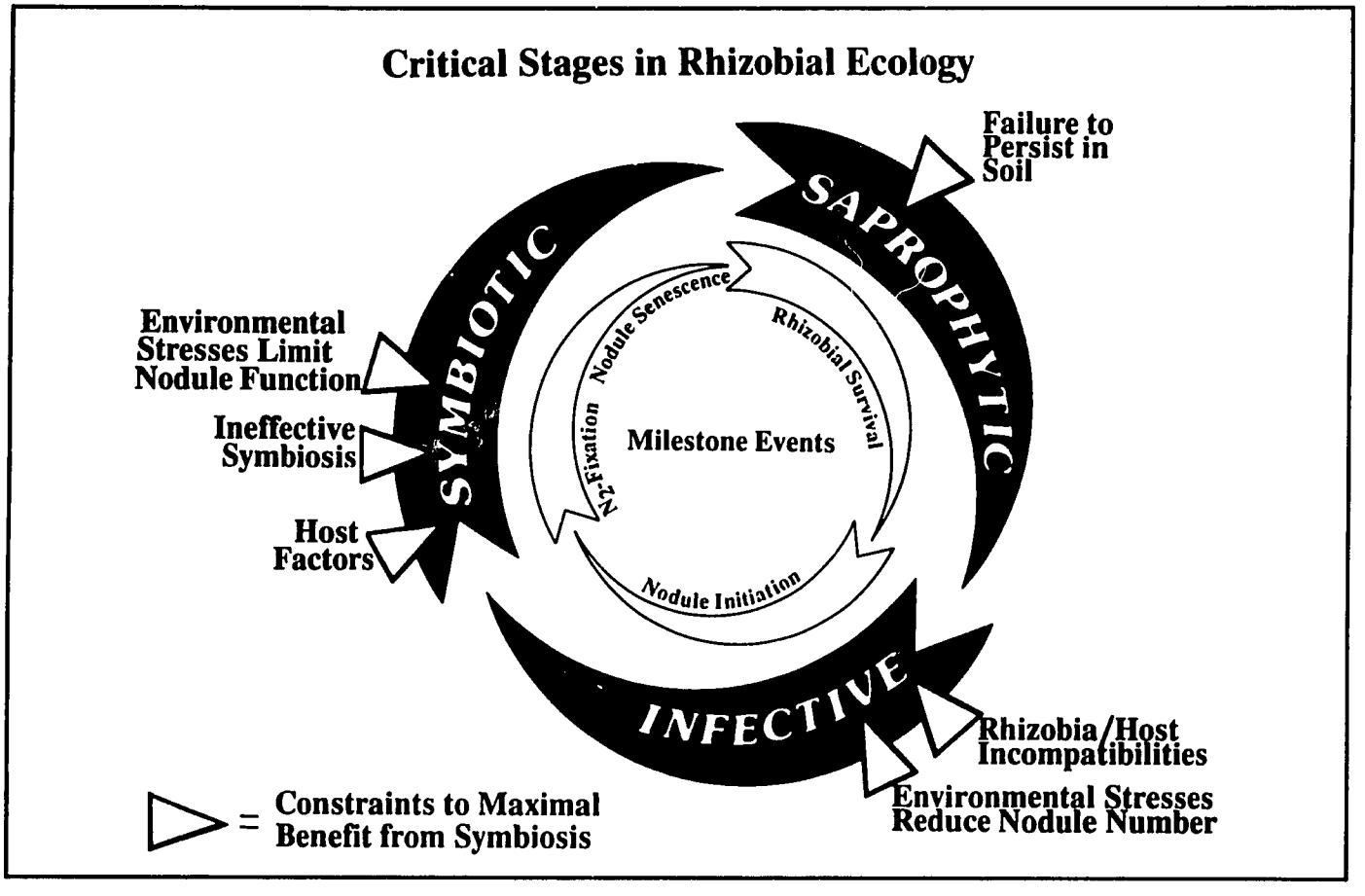
The presence of environmental constraints to BNF is almost universal in farmers' fields. Any environmental stress acting on any of the complex processes of the symbiosis will result in reduced amounts of N_2 fixation and yield. The effects of soil stresses such as low P fertility, salinity, drought, and acidity can directly affect rhizobia in the soil, in inoculants, or at infection sites on plant roots. These same stresses also may affect root growth, susceptibility of infection sites, and plant photosynthetic capacity which reduces energy sources for the reduction of N_2 in root nodules.

Environmental stress may also come in the form of other microorganisms competing for resources on the root surface and competing for infection sites at the expense of our selected, highly efficient strains. Even soil N availability may be considered a stress upon the symbiosis since the assimilation of mineral N substitutes directly for fixed N and legume culture can therefore deplete soils of N under certain circumstances.

Understanding environmental impacts on all components of the BNF process is a prerequisite to identifying problems and developing management strategies for their solution at the farm level. The highly integrated nature of the symbiosis and complexity of environmental factors affecting the symbioses requires developing a multifaceted research agenda. Developing models which quantitatively describe the impact of various environmental and management factors on the performance of legumes is one way to make data more useful across many environments.



The field layout is critical in experiments. This field clearly shows the difference between legume type and experimental treatment.



This conceptualization of the critical stages in rhizobial ecology simplifies the complex elements that need to be understood to take full advantage of the legume/rhizobia symbiosis.

Rhizobial ecology in tropical pasture systems

An important benefit derived from the presence of legumes in pasture systems is the N contributed through BNF. The success of the root nodule symbiosis in many pasture systems is largely dependent on the ability of rhizobia to persist in fallow soils and within the rhizospheres of non-host plants. Such factors as moisture stress, high temperatures and soil chemical factors such as low pH and the accompanying toxic levels of aluminum, iron or manganese all contribute to the fate of rhizobia in soils. A greater understanding of the ecology of rhizobia in soil will contribute to better utilization of the legume symbiosis in pasture improvement efforts.

We have studied the ecology of rhizobia in the diverse climates and vegetation of Maui, Hawaii. Many of the sites represent predominant tropical systems including short and tall grass savannas, and upland and lowland grassy pastures. The population size of the rhizobia in the soils and rhizospheres of these pastures is correlated with environmental factors. The importance of the legume component on rhizobial population is established. The frequency of observing both associated legumes and rhizobia at a site is .94. The density of legumes correlates significantly with the density of rhizobia in soils. Conceptual and regression models were derived which predict the population size of rhizobia in tropical pasture systems and which describe the environmental influences on native and introduced rhizobia in the soil supporting host and non-host swards.

Selection of rhizobia for persistence in tropical soils

Rhizobia known to be highly effective on their appropriate hosts were introduced into diverse tropical soils and the population monitored for 12 months. Once released into the environment, the introduced populations rapidly declined until survival equilibrium was achieved. The microbial densities of individual strains at survival equilibrium were related to the properties of the soil, ranging between <3 to 31,000 cells/g of soil. Individual strains within a rhizobial species were shown to be better adapted to specific stressful environments.

Selection of inoculant rhizobia

	torrid (3)	fertile (9)	weathered (5)
B. japonicum			
USDA 110	*	**	**
USDA 138		**	*
CB 1809		**	**
B. species			
Nit176 A22	**	**	**
TAL209	**	**	*
CIAT 71			*
R. species (Leucaena)			
TAL 82	**	**!	
CB 81			
CIAT 1967		**!	**

* 25-99 cells/g soil after 1 year

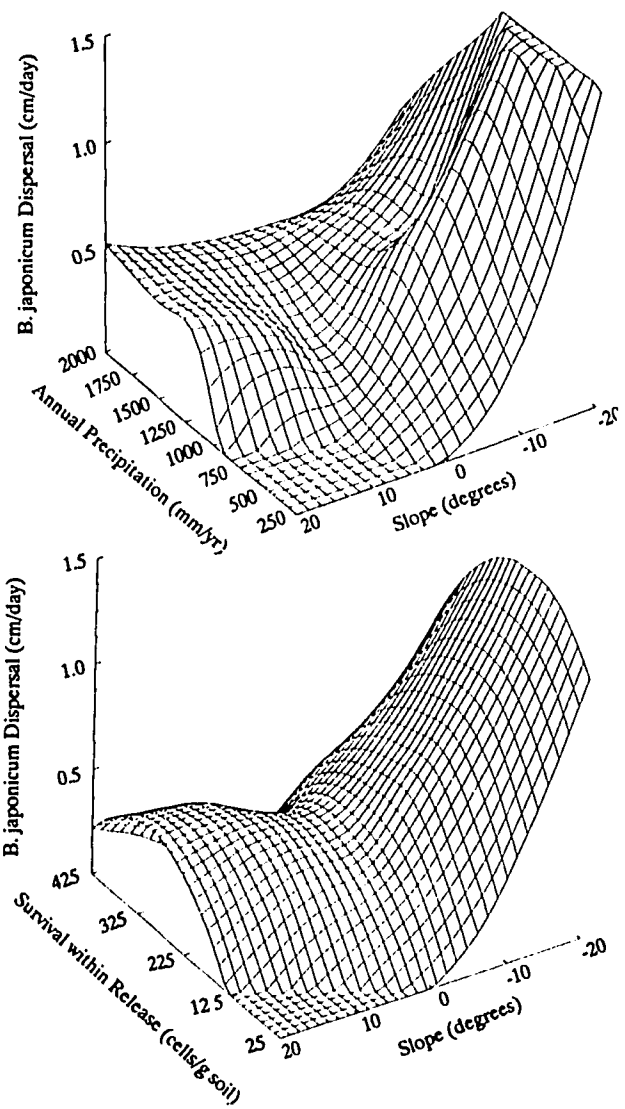
** >100 cells/g soil after 1 year

! introduction outnumbers indigenous rhizobia

There were significant genotype by environment interactions between individual strains within a single environment and by the same strains in different environments. Regression models which predict the microbial density at survival equilibrium for different rhizobial species and strains across a wide range of tropical soil conditions accounted for up to 69% of the observed variation. The results describe the ecological amplitudes of inoculant quality rhizobia and may be used to target these rhizobia to specific environments.

Dispersal of introduced rhizobia in tropical soils

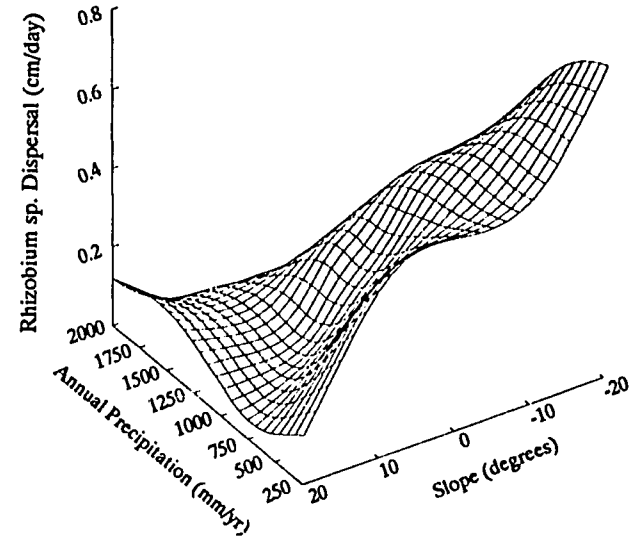
The ability to predict the movement of introduced microorganisms in soils allows for greater understanding of the environmental impact following the release of these organisms. This study was conducted to measure the rate of dispersal of introduced *Bradyrhizobium japonicum* and *Rhizobium* sp. (*Leucaena leucocephala*) under a range of soil conditions. Rhizobial species were released into four soils at the

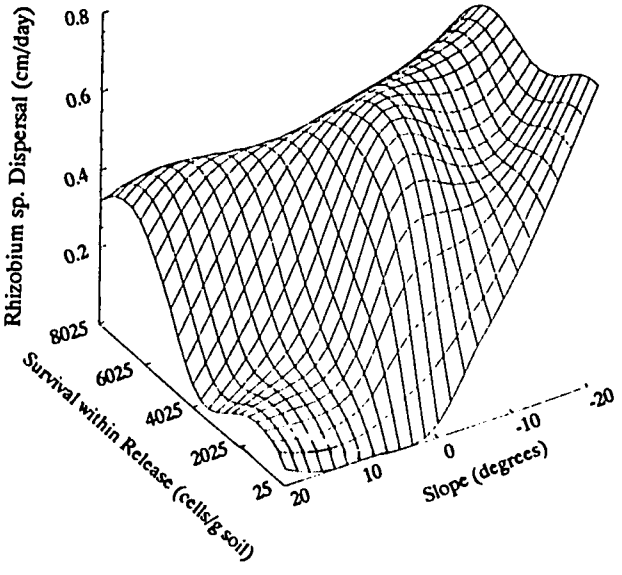


Rate of dispersal (cm/day) of introduced Bradyrhizobium japonicum as influenced by annual precipitation, slope, and the survival within the release site.

rate of 450,000 cells/spp./g soil. After 72 weeks, the population sizes were measured using plant infection techniques along transects which accounted for slope, wind direction and distance from the original release site. The rates of dispersal ranged between 0 and 357 cm/yr, depending on soil conditions.

Slope had the most important influence on the dispersal of the introduced rhizobia. Other controlling factors include the size of the source population and mean annual rainfall (mm/yr).

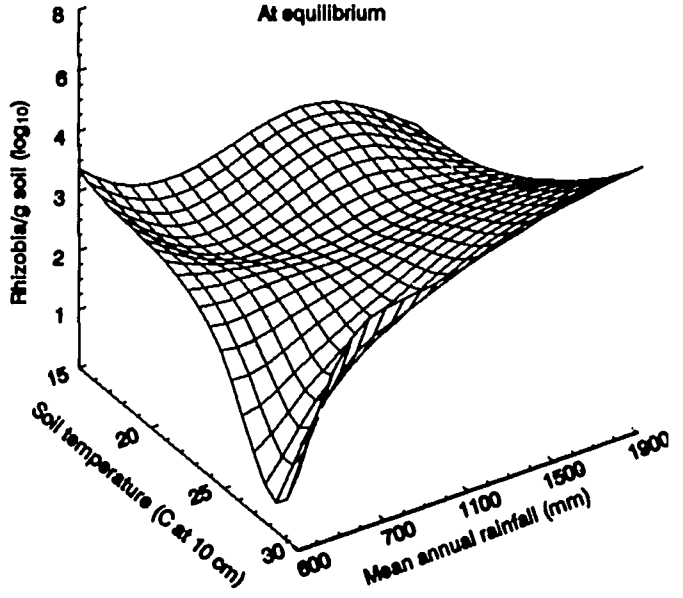


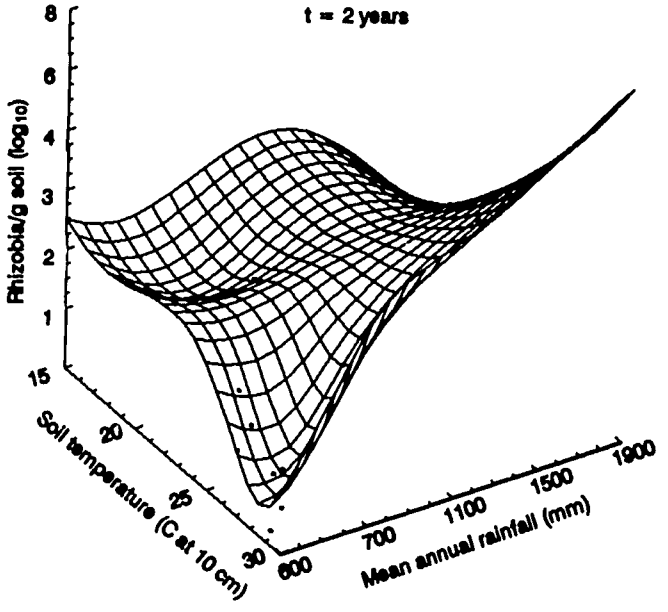


Rate of dispersal (cm/day) of introduced Rhizobium sp. as influenced by annual precipitation, slope, and the survival within the release site.

Modelling the survival of introduced rhizobia in soils

Assessment of the environmental impact of released microorganisms requires the ability to predict their fate based on measurable ecological parameters. A 2-year study was conducted to follow the survival of 18 strains belonging to six species of *Rhizobium* and *Bradyrhizobium* introduced into 18 diverse field sites on the island of Maui, Hawaii.





Persistence characteristics of introduced Bradyrhizobium japonicum in tropical soils: Population changes at fourteen sites over two years.

Peat-based inoculant was applied at the rate of 1.5 x 10^5 cells/strain/g soil. The survival of the bacterial species was monitored using plant-infection procedures; specific strains were followed by serotyping the resulting root nodules. The sites were kept fallow for the duration of the experiment. Populations of introduced rhizobia underwent a rapid decline upon release, followed by either survival equilibrium or complete disappearance depending on strain and/or site characteristics.

The survival of *B. japonicum*, *R. trifolii*, and *Rhizobium* sp. (Leucaena) over time were analyzed using non-linear regression techniques.

The Mitscherlich model, $y = A + [B*Exp^{(C*time)}]$, described the populations over time with the greatest frequency where A = the population size of the introduced rhizobia at survival equilibrium; B = the size of the released population (the increase above A at time 0); and C = the kinetic decline constant. When the values of the coefficients A and C were regressed against a data set which describes the soil, climatic, and biotic characteristics of the sites, components of the environment which co-vary significantly with the Mitscherlich coefficients were identified.

These results provide a useful model for assessing survival kinetics of introduced microorganisms in soils and identifying the key components of the soil environment which influence survival of specific bacteria.

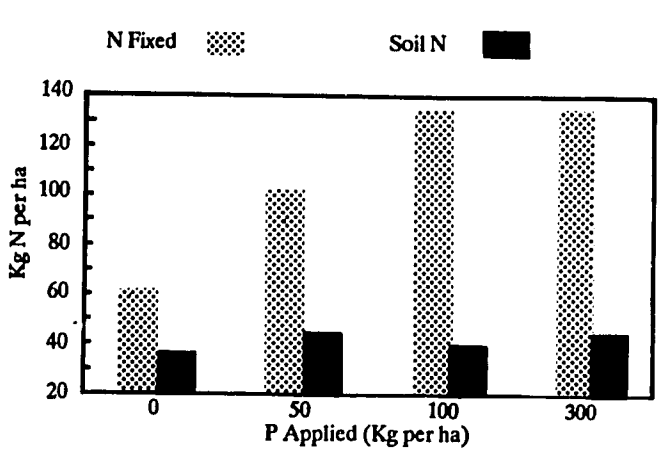
Phosphorus management and soil nitrogen dynamics in a P fixing tropical Ultisol

The following figure shows how nitrogen fixation and soil N uptake by soybean varied with P management. Soil N uptake by this crop was insensitive to P management while nitrogen fixation was increased significantly with improved P fertility. Under conditions of low P fertility, therefore, N from soil resources formed a much larger proportion of total crop N than when P was applied. Only when nitrogen fixation was enhanced with P fertilization did the N balance of the system approach a positive value.

Large areas of the tropics have highly weathered infertile soils. A major constraint to crop productivity is low phosphorus availability. The problem is compounded since these soils are not only low in P but the use of phosphatic fertilizers is often made inefficient. Many tropical soils adsorb P so strongly that applied P becomes relatively unavailable to the plant. Understanding how the farmer may derive the greatest benefit from his investment in P resources is therefore extremely important.

While legumes derive their N requirements from the atmosphere, they still must exploit soil resources for other essential elements. Nitrogen fixation by legumes would be limited without adequate amounts of these essential nutrients. Although nitrogen fixation by legumes is a low input technology and a necessary component to sustainable agriculture, the symbiosis must be managed efficiently if its full benefit to the crop system is to be realized. NifTAL, in collaboration with the University of Hawaii IBSNAT Project and the University of California, Davis, Department of Agronomy and Range Sciences, has initiated a research program to examine the effect of the interaction between P management and inoculation on N and P input-output models in a crop system. This multiple year study will develop response surfaces for crop yield in terms of P application and nitrogen fixation. The dynamic processes of both P and N availability in the soil and fate of soil organic matter will be monitored. Results will indicate some of the necessary management interventions required to sustain legume yield and soil productivity in these problem soils. The approach may have implications for defining strategies in general to sustain yields in soils that become more intensely cropped over time.

Effect of P on N_2 Fixation and Soil N Uptake by Soybean at Kulaha



Data of soil organic matter, organic P and N, and extractable P are being collected and will provide additional insight on how management and nitrogen fixation affect the sustainability of soil fertility.

Influence of temperature and applied nitrogen on rhizosphere colonization, nodulation and competition by different rhizobial strains on field-grown soybean and common bean

Soybean (*Glycine max* (L.) Merrill) and common bean (*Phaseolus vulgaris* L.) inoculated with peat-based rhizobia, were grown with three nitrogen levels at 320m and 1050m sites along an elevational transect on the island of Maui.

Site differences and nitrogen treatment had no significant effect on rhizosphere colonization or nodule occupancy of the three strains. In soybean, strain TAL 102 (USDA 110) occupied the majority of the nodules, while strain TAL 379 (USDA 138) was the least competitive in nodule formation in all treatments. In common bean, TAL 182 outcompeted the other two strains in nodule formation while TAL 1797 (CIAT 899) formed the least number of nodules. Nitrogen application and site location had a significant influence on nodule number and mass for both legumes. This work was done in collaboration with Robert Abaidoo of Ghana who spent a year at NifTAL on an International Atomic Energy Agency (IAEA) Fellowship.

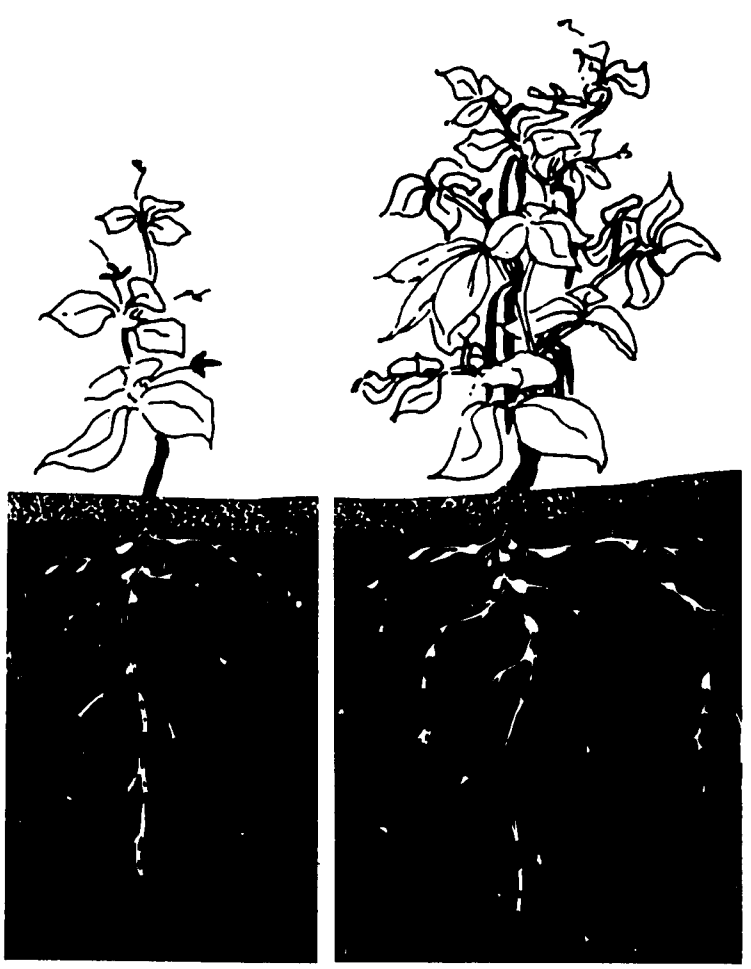
Robert Abaidoo examines soybean roots. The nodulation in the plant at right provided sufficient nitrogen for an abundance of seed pods.

Biological Constraints to Maximal Benefit from Legume Symbiosis

The basics of understanding why rhizobia does not necessarily increase legume efficiency is found in the complex relationship between the legume host and the rhizobia themselves. Over the years, NifTAL scientists have studied these relationships, using field trials, laboratory and green house studies, and most recently biotechnological and genetic techniques. Some other exciting and useful techniques use hydroponics and split root systems to better control the experimental variables.

Predicting a response to inoculation or understanding why different elements increase or inhibit nodulation is also a part of understanding this relationship.

Although the specifics of host-strain interaction which ultimately determine the potential of the symbiosis is not well understood, this exciting area of research holds the promise that eventually farmers will be able to enhance BNF in their fields and thereby increase crop production.



Matching the right rhizobia with the right host yields vigorous plant growth.

High temperature effects on symbiotic plasmids in peat inoculants

Symbiotic genes for nodulation and nitrogen fixation are harbored on the symbiotic plasmids of many *Rhizobium* sp. Without the physical presence of the symbiotic plasmid, the *Rhizobium* will not nodulate the host legume. The influence of high temperature and moisture stress on the symbiotic plasmids of *Rhizobium* sp. was investigated with strains carrying Tn5 insertions. Analysis by agarose electrophoresis showed that *Rhizobium* sp. do not physically lose their symbiotic plasmids in peat inoculants exposed to high temperatures and moisture stress.

Genus-specific microbial regulation of leghemoglobin (Lb) expression in root nodules of interspecific hybrids of Phaseolus

Analysis of the nodule Lb electrophoretic profiles of seven interspecific hybrids showed the expected Lbs and Lbf pattern for five of the hybrids. Lines H/4 and H/5 expressed only the Lbf form, confirming the suggestion that these lines might be diploid and not true hybrids (L. Lewinson, personal communication). Hybrid H/7 was selected for more detailed analysis as it was the most inbred of the hybrids for which seed was available.

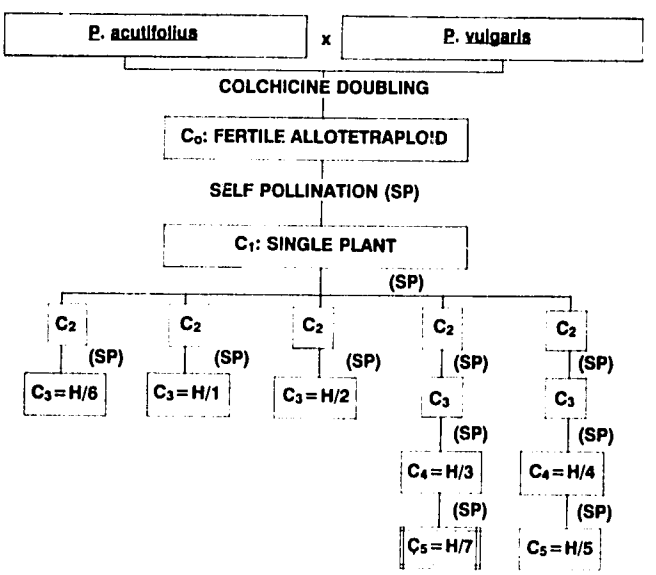
Occupancy of individual root nodules of P. acutifolius, P. vulgaris and their interspecific hybrid (H/7) inoculated with R. leguminosarum bv. phaseoli (TAL182) or Bradyrhizobium spp. (TAL644) alone and in mixtures.

	Nodule source								
	P. acutifolius inoculated with:			P. vulgaris inoculated with:			Hybrid H/7 inoculated with:		
Fluorescent antibody	182	644	182+644	182	644	182+644	182	644	182+644
TAL 182	-[a]	0[b]/5[c]	0/2	4/4	-	1/1	-	0/12	0/7
TAL 644	-	5/5	2/2	0/4	-	0/1	-	12/12	7/7

[a] : no nodules
[b] : number of nodules showing positive immunofluorescence
[c] : total number of nodules examined for immunofluorescence

GENEALOGY OF PHASEOLUS INTERSPECIFIC HYBRIDS

Crosses were originally produced at CIAT, Colombia and seed supplied to the NifTAL Project by L. Lewinson. The pedigree shown below was developed by information supplied by L. Lewinson with the seed. Hybrid H/7 was the primary seed source used in the analyses reported in this communication.



Nodules used for Lb profile analysis were typed for strain occupancy using the fluorescent antibody technique. The accompanying table summarizes that data which clearly shows that the specificity of the two parental lines for their strain requirement in these combinations and that the hybrid (H/7) has inherited the susceptibility pattern of the *P. acutifolius* parent. No instances of mixed infection were observed nor was there evidence for cross-inoculation of the parental lines and strains throughout these experiments.

Data for the more detailed analysis of H/7 in a small pot experiment and using the split-root system are displayed in the figures at left. The hybrid contains the genetic information to synthesize both Lbs and Lbf. When exposed to TAL 644 alone, H/7 responds by producing predominantly Lbs ("*acutifolius*" Lb) and a small amount of Lbf ("vulgaris" Lb).

The presence of TAL 182 in the inoculating mixture, or on the opposite side of the split root system, results in more uniform synthesis of both components and in some cases in production of higher amounts of Lbf depending upon the specific plant and nodule analyzed. These data clearly suggest that the signal to turn on the Lb genes in the hybrid is influenced by the infecting rhizobial strain and that, although the presence of rhizobial strain TAL 182 is required for full expression of the "vulgaris" Lb, the formation of a nodule by that strain is not required. Such an interaction attributes a greater role for the microbial component of the symbiosis in Lb gene expression than has previously been demonstrated. This system provides unique experimental material for the continuing analysis of Lb gene expression.

This work was done by visiting scientist Brian Holl of the University of British Colombia, Canada.

The Rhizobium requirements of Gliricidia sepium - a fast growing nitrogen fixing tree legume

G. sepium is a fast growing nitrogen-fixing tree legume. This species is presently receiving much attention because of its high potential in reforestation and alley cropping. In many tropical countries, this species is fast replacing *Leucaena leucocephala* because unlike *L. leucocephala*, *G. sepium* is resistant to psyllid attack. Recent work done at NifTAL on the rhizobial requirements of *G. sepium* indicated that it is nodulated effectively by fast-growing *Rhizobium* spp. from *L. leucocephala*, *Acacia pennatula*, *Pithocellobium dulce*, *Prosopis pallida*, and *Calliandra callothyrsus*. Several effective strains of *Rhizobium* have been identified as potential inocula for inoculating *G. sepium*.

These strains were tested on six provenances of this tree legume. Results showed that the provenances differed significantly ($p<0.001$) in their shoot

dry matter production and nodule dry weight. However, the strains of *Rhizobium* were significantly ($p = 0.01$) different only for nodule dry weight. The provenance trial indicates that high yields of *G.*

Gliricidia sepium tops and nodulated roots.

sepium can be achieved by selecting provenances with high yield potential and inoculating them with effective strains of *Rhizobium* spp. In a follow up greenhouse experiment, *G. sepium* was tested for inoculation response in three tropical soils. *G. sepium* responded to inoculation in one soil where native rhizobia for *G. sepium* were absent. Highly effective rhizobial strains have been identified for *G. sepium* and are presently being used to produce peat-base inoculants to supply various projects and programs experimenting with this promising fast growing tree.

Multiple forms of glutamine synthetase in nodules of tropical legumes inoculated with Bradyrhizobium spp. and Rhizobium spp.

The profiles of glutamine synthetase (GS) activity in the plant fraction of nodule extracts from tropical legumes, inoculated with either fast- or slow-growing rhizobia, were analyzed by non-denaturing polyacrylamide gel electrophoresis. The electrophoretic mobility of nodule GS was plant dependent but was virtually unaffected by the type of *Rhizobium* present in the nodules. Only *Macroptilium atropurpureum* (DC.) Urb. nodule extracts displayed two distinct GS activity bands similar in electrophoretic mobility to those previously reported in *Phaseolus vulgaris* L. nodules. Nodule extracts of *Vigna unguiculata* (L.) Walp. exhibited one major and one minor band of GS activity. In contrast, nodule extracts of *Glycine max* (L.) Merr. and *Sesbania rostrata* Brem displayed a single zone of GS activity. These experiments indicate that multiple GS activity bands in root nodules are not restricted to *P. vulgaris* and *P. lunatis* L., but are probably of wide spread occurrence in legumes.

Crop growth pattern and efficient nitrate assimilation: two factors that may constrain nitrogen fixation By Phaseolus vulgaris

Phaseolus vulgaris or common bean is one of the world's most important legumes that is directly consumed by humans. Common bean is a major source of dietary protein for farmers and urban dwellers in Latin America and the highlands of Africa and is gaining increased acceptance in Asia. Despite its desirability as a pulse, the common bean has—in the scientific community—a reputation for fixing low amounts of nitrogen.

NifTAL has undertaken a comprehensive research program to evaluate mechanisms related to mineral N assimilation that condition the amount of N fixed by *Phaseolus*. Field experiments were conducted to evaluate the synchrony of demand for nitrogen (plant development and growth) and development of the symbiosis. These field experiments also compared the relative degree to which common bean and soybean relied on soil N to meet crop nitrogen requirements. Results obtained at NifTAL and in the international experimental network indicated that *Phaseolus* may be more efficient at mineral N assimilation than soybean. A companion set of experiments was designed which definitively identified efficient nitrate assimilation as a mechanism involved in reduced nitrogen fixation by *Phaseolus*. Results of this research provide information to plant breeders and agronomists with which to

develop strategies to enhance and fully exploit nitrogen fixation by this important crop. Following are descriptions of the experiments, the results, and implications for improving *Phaseolus* yield.

Paul Singleton and Thomas George are pleased with the growth of plants in this field experiment.

Relation between N assimilation characteristics and N_2 fixation by soybean and common bean

A field experiment was conducted to understand the relationship between crop N requirement, N assimilation characteristics, and N^2 fixation by soybean and common bean. N requirement and soil N availability were manipulated by growing soybean and common bean at elevations of 320m and 1050m with mineral N application of 9, 120, or 900 kg N/ha. Ammonium sulfate with an excess of ^{15}N-isotope was used as the mineral N source. N assimilation by both legumes increased by 25% by application of 900 kg N/ha compared to the 9 kg N/ha. On average, soybean assimilated 48% more N than common bean and because of this, N assimilation by soybean responded more to N application than common bean. Soybean and common bean were found to have distinct N assimilation patterns over time. On average 50% of the total N assimilated by common bean occurred during vegetative growth, the period of which accounted for 56% of its total crop duration, indicating that growth and N assimilation were proportional.

In contrast, only 28% of its total N was assimilated by soybean during its vegetative phase, a period that accounted for 44% of its total crop duration. The slow rate of N assimilation by soybean during the vegetative phase was followed by a period of rapid N assimilation in the early reproductive phase (flowering and pod set) when 56% of the total N was assimilated in 24% of its total crop duration. Nitrogen fixation by soybean also reached a maximum during the early reproductive phase, indicating the synchrony between peak crop N demand with peak N_2-fixation. Isotopic analysis indicated that the early N demand of common bean was mostly met by soil N assimilation. The mineral N use efficiency of common bean was about two times higher than soybean at flowering indicating greater efficiency in soil N assimilation by common bean during early growth; thereafter, mineral N use efficiencies were similar between the two legumes. Consistent with the higher mineral N use efficiency of common bean during vegetative growth, the extractable soil N was significantly lower in plots of common bean than soybean. Maximizing N derived from fixation in common bean may require altering its N assimilation characteristics through plant breeding.

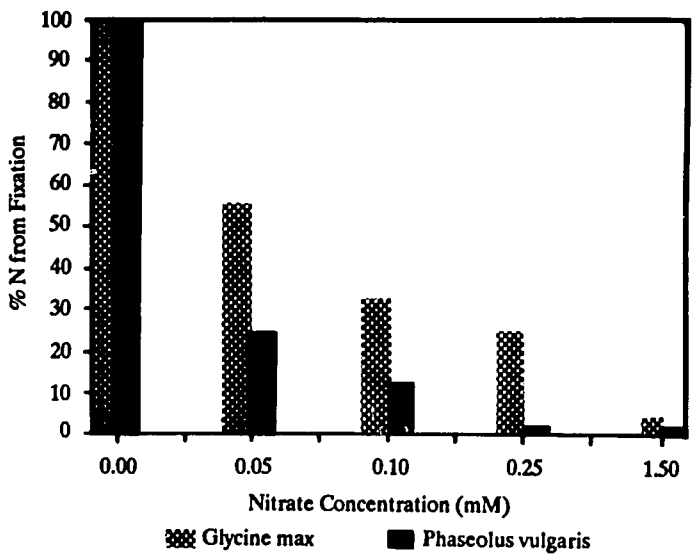
The Relationship Between Root Morphology and Nitrate Assimilation by Phaseolus vulgaris and Glycine max.

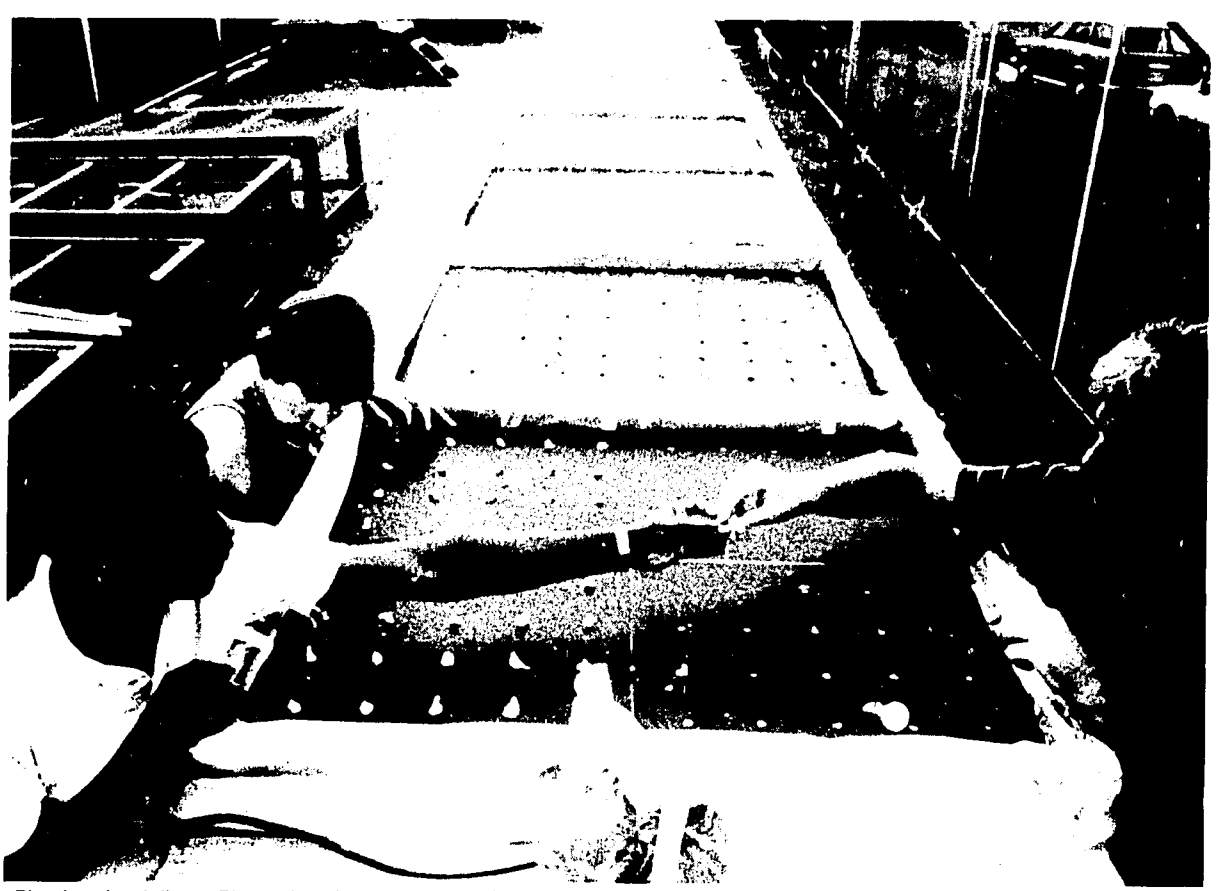
Results from field experiments indicated that under identical conditions common bean derived more of total crop N from the soil than soybean. A series of solution culture experiments were designed to identify mechanisms that may be involved in this phenomena.

In one experiment, both common bean and soybean were grown in solutions with 0.00, 0.05, 0.10, 0.25, and 1.50mM NO_3 that were enriched with ^{15}N. The ^{15}N enrichment in these controlled conditions permitted a precise measure of nitrogen fixation by the two species. The figure below demonstrates that NO_3 had a more adverse effect on nitrogen fixation by common bean than soybean. With only .05m M NO_3 in solution nitrogen fixation by common bean was reduced by 75% compared to the 0.00 control while soybean fixed more than 50% of its total nitrogen at this N concentration. *Phaseolus* assimilated significantly more nitrogen than soybean from both symbiotic and mineral sources indicating that it is not low N demand that results in lower nitrogen fixation by *Phaseolus* in this growth system. Measures of specific root length (root length per unit root dry weight) indicate that common bean has a greater root surface area per unit of root than soybean.

Another experiment was conducted to measure the flux of NO_3 into the root of each species. Both species were grown in solutions of 0.05mM NO_3 at natural abundance levels of ^{15}N then transferred to solutions with 99atm % ^{15}N for two hours. Results demonstrate that common bean assimilates NO_3 at five times the rate per unit root weight or per unit root length than soybean. Therefore under a constant concentration of NO_3 *Phaseolus* assimilates mineral nitrogen more efficiently per unit carbon partitioned to roots than soybean. This phenomena provides scientists with at least one measurable mechanism affecting nitrogen fixation that can be considered when developing a strategy to improve nitrogen fixation by *Phaseolus vulgaris*. Increasing the dependence of *Phaseolus* on symbiotic sources of nitrogen may not only enhance yields but reduce the depletion of soil nitrogen resources that can better benefit the yield of non-leguminous crops.

Percent of Shoot Nitrogen from Fixation as Determined by N-^{15} Isotope





Planting the delicate Phaseolus shoots. Thomas George (from left), Janice Thies, and Kevin Keane cushion shoots with cotton and suspend the root through floating styrofoam sheets into the nutrient solution.

Models for predicting legume response to rhizobial inoculation

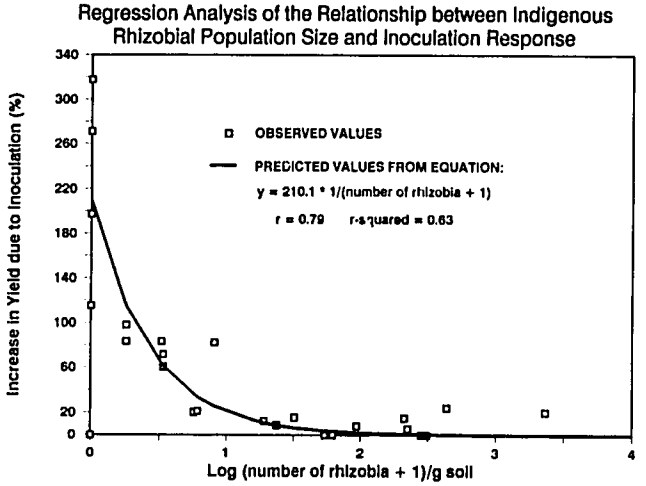
Soil physicochemical constraints, climatic, and biological factors all condition plant response to inoculation. A 3-year study was undertaken to determine which environmental factors best predict locations where inoculation with rhizobia will result in increased crop yield. Field trials with nine legume species were planted under three N source regimes (uninoculated, inoculated, and fertilizer N) at 5 ecologically diverse sites on the Island of Maui, Hawaii.

Site soils differed in N availability and numbers of indigenous rhizobia capable of nodulating the nine legumes. Inoculation response was measured as percent increase in grain yield, biomass, and N content of inoculated over uninoculated plants. Percent difference in yield between fertilized and uninoculated plants served to measure the soil N deficit for each legume species at each location. Non-nodulating soybeans planted at each site provided a biological measure of soil N availability and allowed for the determination of N derived from nitrogen fixation. Increased nodule number and mass, and percent of nodules occupied by inoculant strains indicated success in inoculum establishment. Response to inoculation and nodule occupancy by inoculant strains were found to be inversely related to the number of indigenous rhizobia. As few as 50 rhizobia/g soil attenuated or eliminated inoculation response.

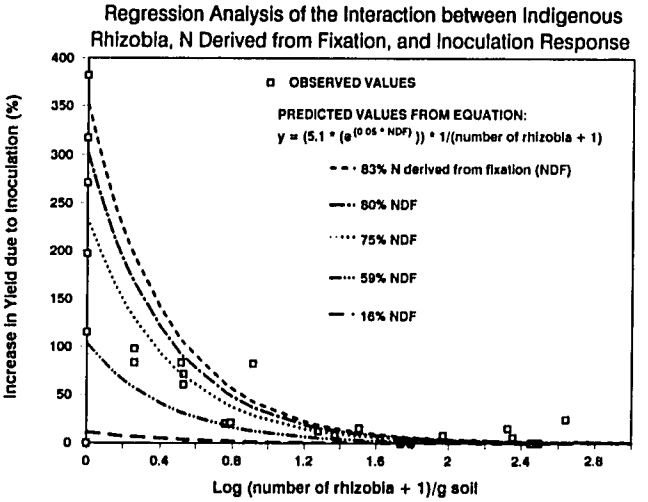
A minimum doubling in nodule mass and 66% nodule occupancy by inoculant strains were required to significantly increase yield of inoculated over uninoculated plants. These results provide a measure of the tremendous barrier to performance of inoculant strains that native rhizobia represent and emphasize the importance of evaluating native rhizobial population size as a part of any comprehensive inoculation program.

Mathematical models were subsequently developed which can predict the likelihood of obtaining a yield response to rhizobial inoculation with high probability. Models couple indigenous rhizobial population estimates with measures of soil N deficit, soil N availability, and N derived from N_2 fixation. While models based on the post-harvest variables, soil N deficit and N derived from N_2 fixation, are best correlated with actual yield increases due to inoculation ($r = .90$ and $r = .96$, respectively), the model based on preplant estimates of soil N availability also correlates well with inoculation response ($r = .83$). This model has the added advantage of being truly "predictive." These results demonstrate that the magnitude of the indigenous rhizobial population coupled with soil N availability in relation to host demand for N are of primary importance as predictors of inoculation response. Measurement of these environmental variables should provide the predictive capability needed by regional planners to help them decide whether rhizobial inoculant production facilities are required in a given region, where the facilities should be located, and what their production capacity should be. In this way, developed models may help to assess the financial feasibility of such projects by forecasting the possible return from investments.

Regression Analysis of the Relationship between Indigenous Rhizobial Population Size and Inoculation Response



Regression Analysis of the Interaction between Indigenous Rhizobia, N Derived from Fixation, and Inoculation Response



Methodological Constraints to Better Understanding of Legume/Rhizobia Association

Inoculant production technology is the heart of NifTAL's technology transfer program and, therefore, a critical part of our research efforts must go toward developing the most cost effective, reliable, and efficient process possible.

Appropriate, low and medium-scale production systems have been developed and are being used in several locations, for instance, Burma, Pakistan, Sri Lanka, Thailand, and Zambia. But, providing equipment is not the only task necessary to implement the technology. Part of the process is learning the most effective ways of working with rhizobia.

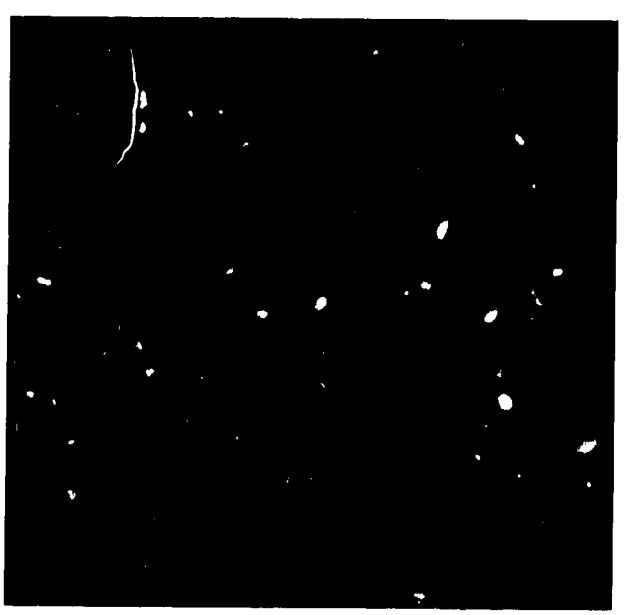
This knowledge can be as sophisticated as developing computer programs to accurately estimate numbers of rhizobia in soil or as basic as developing techniques for screening rhizobia by looking at the chlorophyll content of leaves. The ability to identify differerent rhizobia is a challenge met by identifying and testing suitable serological and genetic methods.

By continuing to test and improve our own inoculants, NifTAL perfects its ability to make recommendations on the most effective strains for a particular legume. As well, looking at different carriers for rhizobia and adhesives for coating seeds provides alternatives that may be more appropriate in a particular locale. The ultimate goal then is to ensure that effective inoculants are available to farmers on a local basis.

All rhizobia work begins with harvesting nodules from plant roots. Wendy Asano carefully removes nodules in preparation for counting, weighing, and serotyping.

Monoclonal antibody separation of B. japonicum serocluster 123 into subgroups based on specific and shared somatic antigens

Members of Serogroup 123 of *Bradyrhizobium japonicum* are present in many soils of the soybean growing regions if the U.S. and prevent establishment of more effective inoculum strains in nodules of soybeans grown in the field. This serogroup consists of a diverse group of strains known as Serocluster 123, all of which react positively with polyclonal antiserum against the type strain USDA 123. Monoclonal antibodies (MCAs) were developed against USDA strains 123, 127, 129, and PRC 83, all belonging to Serocluster 123. Eight MCAs, 7 IgM and 1 IgG, were selected because of their ability to identify specific and shared antigens on each of the strains. One MCA was specific for each of the immunizing strains, and four reacted with two or more of them. A fluorescent antibody made with MCA specific against strain 123, reacted strongly with that strain and not with any of the others. Based on the pattern of radioimmunoassay reactivity with the 8 MCAs, we were able to group 99 isolates from diverse geographic regions into at least 20 groups and subgroups. In this study, MCAs revealed a large degree of heterogeneity in Serocluster 123. This work was done in collaboration with Albert Benedict and Carla Mizumoto of the University of Hawaii and E. L. Schmidt of the University of Minnesota.



Reaction of a nodule smear with MCA #8 FA—only about 25% of the bacteroids stain, and of these, only 1/2 of each cell.

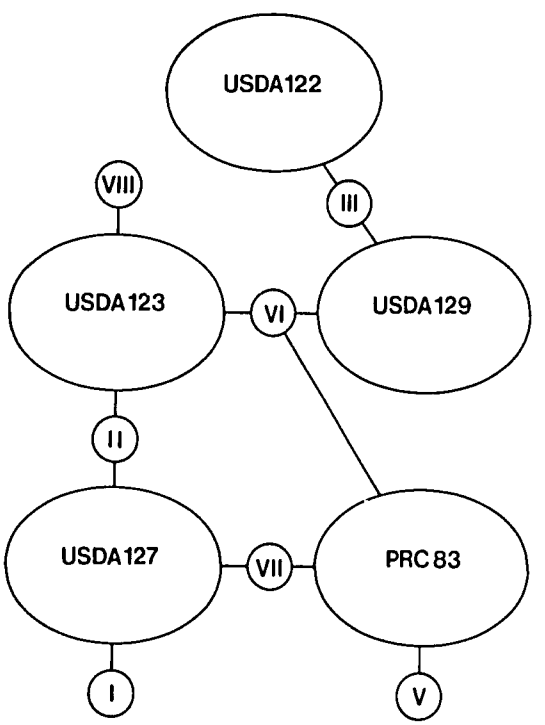
MONOCLONAL ANTIBODIES	PROTOTYPE CULTURES: USDA 123	USDA 127	USDA 129	PRC 83	ANTIBODY ISOTYPE
X1					IgM
X2					IgM
X3					IgM
X4					IgM
X5					IgM
X6					IgM
X7					IgM
X8					IgG 3

NO REACTION

INTERMEDIATE REACTION

STRONG POSITIVE

Patterns of reactivity of 8 MCAs with 4 prototype strains used in immunization.



Mosaic of unique and shared somatic antigens of prototype strains in serocluster 123.

Production of antibodies against serologically recalcitrant strains of Rhizobium sp. of chickpea

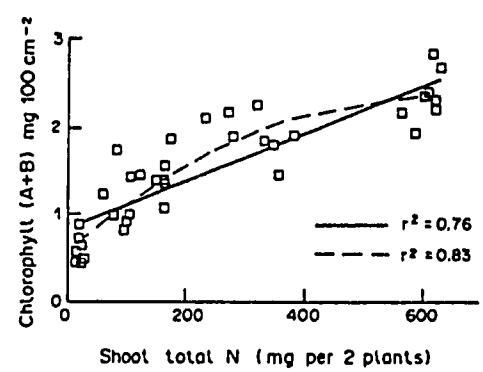
Several years ago, researchers noted that some strains of the chickpea rhizobia failed to elicit a satisfactory immunological response when injected into rabbits. Several attempts showed that this recalcitrance to produce antibodies was strain specific and not due to animal variation. Since some of these strains are efficient N fixers, good quality antisera are required for ecological studies. The aspects of this research that are still continuing in collaboration with IRI scientists are: similarities in intrinsic antibiotic resistances (IAR) between recalcitrant strains; immunoresponse based on different inoculation schedules and antigen concentration; antibody production response to mixed strain immunization; selecting alternative methods for antibody production; and examination of the immunogenicity of different cell fractions.

Non-destructive chlorophyll assay for screening of strains of Bradyrhizobium japonicum

Several direct and indirect methods of measuring the rate and magnitude of nitrogen fixation are employed when strains of rhizobia are screened for effectiveness. The commonly used methods are dry matter production, plant color, total nitrogen content, and nitrogenase enzyme assay by acetylene reduction. Recent investigations by NifTAL scientists demonstrated that the chlorophyll content of the leaves of the soybean plant was significantly correlated to the nitrogen content of the leaves, and also other nitrogen fixation parameters such as nodule mass, dry matter production, and nitrogenase enzyme activity in the nodules (see accompanying figure and table). These results were observed in greenhouse and field grown soybean plants inoculated with soybean rhizobia of varying effectiveness or when fertilized with different levels of nitrogen fertilizer.

The chlorophyll method was relatively easy to perform, economical, fast, non-destructive, and sensitive.

Relationship between chlorophyll and shoot total N in 42-day-old glasshouse-grown soybean plants grown with 5 different concentrations of N_2.



Coefficients of linear correlation (r) for chlorophyll contents, growth, nodulation, and nitrogen fixation characteristics of glasshouse grown soybean as affected by inoculation with strains of B. japonicum of varied effectiveness.

	Growth characteristics					
Traits	Shoot Dry wt.	Shoot total N	Shoot N fixed	Nodule dry wt.	Nitrogenase activity	Specific nitrogenase activity
Shoot total N	0.98***	---	---	---	---	---
Shoot N fixed	0.98***	1.00***	---	---	---	---
Nodule dry wt.	0.87***	0.91***	0.91***	---	---	---
TNA	0.92***	0.86***	0.80***	0.60**	---	---
SNA	0.57**	0.50*	0.17 NS	0.22 NS	0.69***	---
Chlorophyll (a+b) mg $100cm^{-2}$	0.89***	0.88***	0.88***	0.85***	0.79***	0.60**

*,**,*** Indicate simple correlation coefficients significant at 0.05, 0.01, and 0.001 levels of probability.
NS Not Significant

Research Associate Bruce Martin performs a routine technique in which nodule smears are stained prior to identification of the rhizobial strain under a microscope.

Serological methods suitable to assess the performance of rhizobia in developing countries

The failure to increase yields through inoculation does not always mean that there was inoculation failure. Without methods to monitor the success of rhizobia in forming nodules on the plant, erroneous conclusions concerning the benefits of inoculation can easily be reached. As research scientists and funding agencies become more sophisticated in evaluating the legume/rhizobia symbiosis from a holistic perspective, they will require more precise information on the fate and success of rhizobia in the field.

Serological identification of strains of rhizobia is an important tool to follow the fate of rhizobia both in nature and in crop systems. Many methods are available; each with their own set of uses and constraints. Many modern methods have great precision and flexibility of application but require sophisticated materials and equipment. One method that offers both sensitivity for detecting rhizobia and relative simplicity of operation and material requirement is the enzyme linked immunosorbent assay (ELISA). Recent advances with ELISA methods utilized solid filter material as a binding substrate for the antigens and made these procedures even more suitable for laboratories not equipped with advanced equipment. These modifications of ELISA are called the immunoblot method.

NifTAL is actively evaluating a variety of critical reagents for their reliability and more importantly their ability to withstand shipping in tropical environments. The goals of this effort are threefold: 1) provide information to research and industry workers about the suitability of alternate material that may be more readily available; 2) develop an immunoblot assay kit that can be used to standardize

procedures of a research network studying the ecology of rhizobia; 3) develop an instructional tool to train scientists and industry workers in a practical method to assess the performance of rhizobia in the field.

Single vs. multistrain inoculants

Both single- and multistrain peat inoculants are widely used for seed inoculation. We investigated strain effectiveness and competition for nodulation in single- and multistrain inoculated chickpea, dry bean and soybean grown in soils where N was available or immobilized by addition of bagasse. All three legumes showed no significant differences in shoot dry matter production to inoculation with single or multistrain inoculation in a mollisol (Kehua series) where soil N was available. However, when soil N was immobilized, strain differences became more pronounced. The multistrain inoculation was intermediate or as effective as the most effective single strain inoculation. In the multistrain inoculation of chickpea and soybean but not in the dry bean, rhizobial strain competition patterns were altered by the N-status of the soil. In soybean, TAL 102 (USDA 110) was the most effective and competitive while in the dry bean it was TAL 182. In chickpea, TAL 480 was the most competitive when soil N was available, but all three strains were of similar competitiveness when soil N was immobilized. In another low N mollisol (Waikoa series), inoculation of dry bean with a mixture containing equal numbers of TAL 182, Kim 5, CIAT 899, Viking 1 and TAL 1383, showed no significant differences between the multistrain inoculant and single strain inoculation with TAL 182. TAL 182 was most competitve and formed 43.5% of the nodules.

Most Probable Number Enumeration System (MPNES)

The design of most probable number (MPN) experiments is restricted by the availability and completeness of tables for certain dilution ratio and replicate number combinations. The results from a computer program, the MPNES which generates solutions for population estimates and the confidence intervals (p = 0.05) of MPN data from plant infection counts are presented at right. Tables generated by MPNES agreed with existing tables yet MPNES was able to generate other tables and discrete solutions for design combinations that have not been published. MPNES was also used to generate population estimates from fractional base dilution ratios that increase the accuracy of MPN estimates by decreasing the base dilution ratio, by increasing the number of replicates per dilution or by correcting for constantly inaccurate diluent volumes. The MPNES program also adjusted for inoculation volume and initial dilution rates. In this way, MPN experiments were designed to better measure the organism of interest.

```
Do you need a TABLE or an INDIVIDUAL MPN

(T/I)? I

INDIVIDUAL MPN



Would you like to have your
INDIVIDUAL MPN printed?

(y/n)? Y

Base of dilution ratio is?          3
Number of tubes per dilution is?
Options (y/n)? Y
Inoculation volume in ml's is? .5
Initial dilution is 1 to ? 10

Number of positive tubes at dilution level 1 is? 3
Number of positive tubes at dilution level 2 is? 3
Number of positive tubes at dilution level 3 is? 2
Number of positive tubes at dilution level 4 is? 0
Number of positive tubes at dilution level 5 is? 1
Number of positive tubes at dilution level 6 is? 0


Confidence factor:  2.745862
3   3   2   0   1   0
MPN= 191.1937   P(0.95)= 69.62978 - 524.9915


Do you want another MPN (y/n)?
```

A typical computer screen printout from the MPNES program.

Oils as adhesive for seed inoculation and their influence on survival of Rhizobium and Bradyrhizobium spp.

The most common method of inoculation is seed coating with a carrier based inoculant. A sticker is required to cause the inoculant to adhere to the seed. Ideally, such a sticker must have good adhesion qualities, be non-toxic to the rhizobia and the seed, and prevent moisture loss in the rhizobia through evaporation. Water, sugar, and gum arabic have been used as sticker materials with various levels of success. Vegetable oils have been shown to protect against dessication of rhizobia when used with lyophilized cultures in water-based carriers. This study was conducted to assess several vegetable oils for their suitability as inoculant adhesives for seeds of various economically important legumes.

Presterilized seeds of soybean, peanut, chickpea, and bean were coated with various stickers including water, gum arabic, mineral oil, peanut oil, and soybean oil and then inoculated with peat inoculants. Inoculated seeds were stored at 4°C, 28°C, and 34°C for one, three, and nine days. Samples were counted by the spread plate method. Germination tests were performed on all seeds stored for 3 days at 34°C. Seeds of peanut and soybean were planted in Leonard jars after 3 days of storage at 34°C to test for influence of sticker on nodulation.

Results showed that the oils can be used for inoculation of the legumes tested. Although the oil bound fewer rhizobia to the seed, numbers were similar to those obtained by the gum arabic treatments after 9 days of storage. This was especially true at elevated temperatures. All sources of variation were significant as shown in Table 1. Table 2 shows the survival of rhizobia at 34°C. A significant finding in the experiment was the protective effect of peanut and soybean oils on the survival of chickpea rhizobia on inoculated seeds stored at that temperature. The two oils were superior to gum arabic in preserving larger numbers of viable rhizobia. The germination tests showed that oils had no adverse effect on seedling development and the plants tested in the greenhouse nodulated normally with no difference between treatments.

Table 1. Summary of significances of the sources of variation in the analysis of variance on the survival of soybean, peanut, bean, and chickpea rhizobia on inoculated seed stored at 34°C.

Sources of variation	df	Rhizobia of Soybean	Peanut	Bean	Chickpea
Replications	2	NS	NS	NS	NS
Adhesives (A)	4	***	***	***	***
Storage time (T)	2	***	***	***	***
A x T	8	NS	NS	NS	**

, * denote F-value significant at 0.01 and 0.001 probability levels, respectively.
NS Not significant at the 0.05 level.

The experiment showed that oils have most of the qualities expected of a good adhesive for seed inoculation. They are reasonably sticky, inexpensive, readily available, non-toxic to seed and microbe, and prevent dessication. These characteristics make oils uniquely suitable for application in developing countries where elevated temperatures are a problem and gum arabic cannot be considered because of high cost.

Table 2. Influence of adhesive and storage time on the survival of Rhizobium and Bradyrhizobium spp. on inoculated seeds of various legumes exposed to 34°C.

Adhesive	Soybean 1d[a]	3d	9d	Bean 1d	3d	9d	Peanut 1d	3d	9d	Chickpea 1d	3d	9d
Gum arabic	5.88	5.18	3.69	5.85	5.37	5.08	6.10	5.53	4.73	5.83	4.75	2.73
Mineral oil	5.62	4.99	3.82	5.44	4.91	3.84	5.48	5.26	4.54	5.84	5.00	3.65
Peanut oil	5.64	5.19	3.39	4.62	4.49	3.10	5.40	4.89	4.04	6.20	5.62	4.43
Soybean oil	5.39	5.03	3.58	4.22	3.47	2.10	5.41	4.87	4.42	6.32	5.57	3.86
Water	6.01	4.47	4.10	5.17	4.69	3.89	5.69	5.44	4.56	5.32	4.59	2.99
LSD (0.05)	0.28	NS	NS	0.75	0.40	0.32	0.34	0.34	NS	0..25	0.24	0.41

a Storage time in days
NS Not Significant

Institutional Cooperation for Solving Problems

Collaborative research is an essential part of the effort to get technology to developing country farmers. Three of the main reasons for this are validating the technology, demonstrating the technology, and encouraging in-country research. Collaboration and communication between NifTAL and overseas colleagues is required if NifTAL is to focus research on real problems faced by farmers in developing countries.

By conducting joint projects, NifTAL researchers are able to test hypotheses and research findings that have been experimented upon at our Maui headquarters. Although we are able to use many variables of climate and soil type, collaborative projects validate our research under more realistic conditions.

When experiments are done in developing countries, it offers an excellent opportunity to display the benefits of inoculation to the researchers themselves, educators, agricultural department decision makers, extension workers, and farmers.

The opportunity of collaborative research brings the benefits of BNF to the forefront of agricultural research programs, leads to continuing research and support from national institutions and influences decision makers to promote the use of BNF technology.

Consuelo Estevez Salazar of Equador was one of the 22 researchers who participated in the WREN (Worldwide Rhizobial Ecology Network). She is pictured with her growth pouch greenhouse experiment which was only one part of the comprehensive WREN study.

The Effect of Crop History and Environment on Bradyrhizobial Populations in Karnataka State, India

The benefit from inoculating legumes is often determined by the size of the native rhizobial population. Recent results from research at NifTAL have added some precision to our understanding of how environment determines the size and character of native populations of rhizobia.

Survival of inoculants in the crop system is extremely important to farmers. Once superior strains are introduced, their persistence in the crop system is added insurance against inoculation failure. NifTAL and the University of Agricultural Sciences GKVK Campus in Bangalore, India, began a three-year research program to evaluate how crop system, management, and environment affect rhizobia. An initial survey was undertaken in two distinct agro-ecosystems that sampled four types of crop systems. The crop systems varied in terms of the intensity and frequency of legume cultivation. Results of the preliminary survey are shown in the figure.

The average differences between the environments were not dramatic, however crop history had a large effect on the size of the bradyrhizobial population. Long term cultivation of non-legumes reduced the populations to extremely low levels yet even one crop cycle increased the population size by many magnitudes. The highly weathered and eroded soils of the upland intercrop systems also appeared to support populations lower in number than sequential crop systems in the lowland areas. These results indicate the importance of crop system to the nature of rhizobial populations and point to the need to collect additional data to develop a more precise understanding of the interaction between crop system, environment, and rhizobial populations.

Effect of crop systems on numbers of Bradyrhizoibum sp. in two agroecosystems of Karnataka State

Crop systems	Crops	Years	# of rhizobia /g soil Shimoga	Mandya
Sequence Cropping	Rice/lablab	>6		1,950,542
	Rice/mix pulse	>6	0	0
	Ragi/mix pulse	>6	22	230,327
	Ragi/peanut	>6	50	50
Intercropping	Ragi/lablab	>6	66	199,044
	Ragi/lablab	>6	4,085	197,784
	Ragi/lablab	>6		3,007
	Ragi-rice/ lablab	>6	5,500	
Continuous Cropping	Rice	10	0	
	Rice	10	0	
	Rice	10	6	
	Rice	50		240
	Rice	6		172
	Rice/peanut	50/1		226,449
	Sugar	10	22	1,400
	Sugar	10	11	1,242
	Sugar	21	10	46,993
	Sugar	50		

Shimoga - highly weathered, acid, P deficient
Mandya - less weathered soil, neutral pH, low rainfall

Response to inoculation of soybean that nodulate with the indigenous rhizobia in African soils

In cooperation with scientists at the University of Ghana, a project was developed to test the response to inoculation of a soybean that had been bred to nodulate with indigenous rhizobia. The soybean breeding program at the International Institute for Tropical Agriculture (IITA) had as one of its goals the development of varieties which nodulated with a broader range of *Bradyrhizobium* (slow growing rhizobia common to many tropical species) than varieties from North America. The reasoning that justified this effort had practical and scientific merit. Farmers in Africa seldom have access to inoculants and therefore if varieties were available that did not require inoculation, yields would not be compromised from lack of rhizobia. The potential to breed legumes with a broader range of suitable microsynbionts has profound agronomic and ecological implications for other species of legumes and how their culture may alter the indigenous bradyrhizobial populations.

African researchers and scientists at IITA were successful in identifying genotypes with enhanced nodulation compared to North American types when grown in African soils. These soybean genotypes were labeled freely nodulating varieties. The enhanced nodulation also produced yield increases. The bradyrhizobia that nodulate these varieties also nodulate other wild legumes native to Africa. These bradyrhizobia are a subset of the general population in that not all of the slow-growing population will nodulate the freely nodulating varieties. The use of inoculants on the freely nodulating genotypes has not been well investigated. Results are variable but many reports indicate that the freely nodulating varieties do not respond to inoculation. Some preliminary work done in Zambia and reported by NifTAL and the ZAMARE Project (USAID) indicated that under improved management these varieties responded significantly to inoculation.

One aspect of current farm practices in much of Africa—in addition to the general lack of inoculants—is the low level of agricultural inputs available to farmers. The evidence is clear that any constraint to yield also reduces the amount of nodulation by reducing the crop's requirement for nitrogen. Low inputs and the resulting constraints to yield may have contributed to the failure of inoculants to increase yields on the freely nodulating varieties. NifTAL and scientists from the University of Ghana designed an experiment to compare the inoculation response of an indigenous freely nodulating and a North American variety under two levels of management. Phosphorus was selected as a management variable since it is a nutrient commonly limiting crop yield in much of West Africa.

Collaborative research is most effective when conducted by persons trained in rhizobial work. G.P. Msumali and Sayeda A. Abu Nayib participated in a NifTAL training course held in Nairobi, Kenya.

Results from this experiment are dramatic. The table below shows data for nodule weight of variety Davis (North American origin) and Hernon 147 (a freely nodulating variety). Nodule weight is usually a good indicator of nitrogen fixation and a measure of the amount of rhizobia available to the legume. The African variety produced more than ten times the nodule weight of Davis when there was no inoculation and P was applied. Without inoculation or P there was almost no nodulation on either variety indicating that a severe P deficiency was limiting plant growth. Applying quality inoculants increased nodulation of both varieties by many magnitudes under both low and high P conditions. These results indicate that at some sites the freely nodulating varieties may have insufficient bradyrhizobia available to meet all but the very lowest yield potentials. These varieties should be inoculated at all costs; the potential benefit to the farmer is enormous. It would be unwise based on an assumption that farmers planting freely nodulating varieties have their yields constrained by other factors to such an extent that the small population of indigenous bradyrhizobia is sufficient to meet the crop's nodulation requirements.

Effect of inoculation and phosphorus on nodule weight of two soybean cultivars at Accra, Ghana

Treatment	Davis	Hernon 147
Uninoculated-P	5	4
Uninoculated+P	3	31
Inoculated-P	280	339
Inoculated+P	499	505

-P, +P are 0 and 250 kg P/ha; Davis is a cultivar from North America, Hernon 147 is a 'freely' nodulating cultivar from Zambia bred to nodulate with indigeneous rhizobia in Africa.

Symbiotic and competitive properties of Bradyrhizobium japonicum native to Korean soils

It has often been suggested that effective rhizobia can be found in soils of the centers of diversity or origin of the host legume. Korea is one of the centers of diversity of the soybean and it seems reasonable that effective competitive indigenous soybean rhizobia may be found in Korean soils. To test this hypothesis, Korean soils from 10 diverse locations were investigated for the symbiotic potential of the native soybean rhizobia. The "whole-soil inoculation" bioassay technique developed for alfalfa rhizobia was adopted for testing for soybean rhizobia. Results showed that the "whole-soil inoculation" bioassay was a time-saving method for evaluating the symbiotic potential of native soybean rhizobia in Korean soils. It was also found that Korean soils harbor soybean rhizobia which are highly effective. Some of the Korean strains, besides being as effective as the widely used USDA 110, were able to outcompete USDA 110 in competition experiments. However, USDA 123 was the most competitive of the strains studied on two soybean cultivars (see the table below). This collaborative research was funded by the Korean government and carried out with Ui Gwm Kang of Yeong Nam Crop Experiment Station.

Ui Gwm Kang with research associates (from left) Wendy Asano and Kathy MacGlashan.

Competition for nodule occupancy by strains of Bradyrhizobium japonicum indigenous to Korean soils versus USDA 110 and USDA 123.

		% nodule occupancy							
Competing strains	Soybean[a] cultivar	USDA 110	USDA 123	YCK 117	YCK 141	YCK 213	Mixed[b]	Unidentified[c]	F-Ratio[d]
USDA 110 + YCK 117	J	0.5	–	99.5	–	–	0	0	***
	C	0	–	99	–	–	0.5	0.5	***
USDA 110 + YCK 141	J	23.5	–	–	67	–	8.8	0.7	***
	C	13	–	–	58.5	–	12	16.5	**
USDA 110 + YCK 213	J	2	–	–	–	82.5	0	15.5	***
	C	0.3	–	–	–	99.7	0	0	***
USDA 123 + YCK 117	J	–	78	9	–	–	13	0	***
	C	–	64	23.2	–	–	12.8	0	*
USDA 123 + YCK 141	J	–	99.8	–	0.2	–	–	0	***
	C	–	100	–	0	–	–	0	***
USDA 123 + YCK 213	J	–	92	–	–	7	1	0	***
	C	–	73	–	–	18	9	0	***

[a] J and C indicate Jangbaek and Clark soybean cultivars, respectively
[b] Proporiton of nodules occupied by both competing strains in mixed inoculant
[c] Nodules showing negative reactions against all antisera used
[d] ***, **, * indicate significant differences at 0.001, 0.01, and 0.05 levels of probability, respectively, for competing strains

Temperature, photoperiod and mode of nitrogen nutrition effects on legume phenology

Despite interest in modelling growth, development, and yield of legumes for agrotechnology transfer in the tropics, few data are available from tropical environments. Likewise, the nitrogen fixation component of most legume crop models still remains a black box. The ability to predict the timing of phenological events as well as yield attributes of legumes will be useful in cropping system planning. The suitability of legume genotypes to cropping systems is in part determined by the interaction effects of temperature, photoperiod, and other variables on legume phenology. Recent research at NifTAL indicated that the mode of N nutrition is a particularly important variable influencing phenology of soybean and other legumes.

Experiments were conducted in collaboration with the International Benchmark Sites Network for Agrotechnology Transfer (IBSNAT) Project to examine the interaction effects of temperature, photoperiod, and mode of N nutrition on phenology, N fixation and assimilation, and yield of soybean. Photoperiod control systems to facilitate natural, 14 hour, 17 hour, and 20 hour daylengths were established in the field at elevations of 320 and 660m. Nodulating and non-nodulating isolines of Clark soybean were used for the experiment. Nitrogen fixation was measured by ^{15}N-isotope dilution as well as ureide methods. The results indicate that changes in the growth rates and the phase durations with temperature and photoperiod greatly influenced biomass partitioning within the plant and N assimilation from mineral and BNF sources. Additionally, mode of N nutrition influenced the length of pod-fill duration. The data indicate the importance of the inclusion of mode of N nutrition and plant N status in soybean phenology models. These data along with phenology data generated for several other soybean and common bean genotypes are currently being used as the basis to incorporate the BNF component into legume crop models.

The photoperiod experimental set-up used lights and shade cloth to control the light periods. Several different plants were used in the experiment as shown in the inset photograph at top.

Production and conservation of fixed nitrogen in tropical crop systems: interaction between management and environment

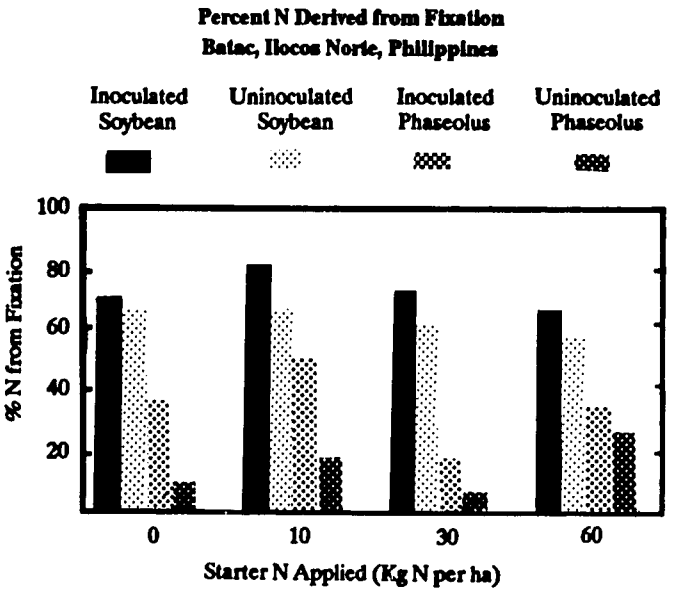
NifTAL and the Philippine Council for Agriculture and Resources Research and Development (PCARRD) through support from USAID have implemented a research program to develop management strategies that enhance nitrogen fixation in crop systems. Two sites with distinct agro-ecosystems were identified that represent frequently encountered management problems in the tropics; an upland site with P deficient soils and an intensely cropped lowland site with a soil deficient in nitrogen and organic matter.

A series of experiments were conducted over a three year period which examined the response to inoculation and nitrogen fixation by four legumes under defined management regimes. The sites' native rhizobial population was characterized so that results from this research program would be relevant to the database being generated in NifTAL's Worldwide Rhizobium Ecology Network (WREN).

The results indicate that specific management strategies can be devised to maximize BNF and legume crop output of each species under realistic levels of inputs. In all cases BNF responded to management inputs which emphasizes the need to consider BNF technology in the context of the whole crop management system rather than as an isolated input. Following are some highlights of this project as it nears completion.

The Need for Starter Nitrogen in Lowland Soils of Ilocos Norte

Through the able assistance of Marianos Marcos State University (MMSU) in Batac, Ilocos Norte, field experiments were carried out to evaluate the extension service's recommendation for applying starter nitrogen to legumes in the region and to determine how these recommendations affected the performance of rhizobial inoculants. Mungbean, peanut, common bean, and soybean were grown with or without inoculation and with either 0, 10, 30, or 60 kg N/ha applied at planting. The current recommendation for starter N is 30 kg N/ha. Isotope enriched fertilizer material was used to measure nitrogen fixation.



The response to inoculation varied with species and was related to both starter N application and the number of rhizobia in the soil. Although soybean and common bean are commonly grown in this area and there were native rhizobia present, the population size was insufficient to meet these crops' N requirements. The crops responded to inoculation. Rhizobia for the other species were only slightly above threshold values where additional rhizobia are required through inoculation to reach crop yield potential.

The bar chart below demonstrates that starter N had a different effect on common bean compared to soybean. While inoculation dramatically increased yield of both species, only common bean responded to the starter nitrogen application. Even with successful inoculation, common bean increased yield with each addition of starter nitrogen. The response to inoculation was enhanced with starter nitrogen inputs to common bean.

Isotope enrichment data of the plant tissue (data not shown) indicated that common bean had a significantly greater fertilizer use efficiency than the other three legume species. This data is consistent with data gathered in Hawaii and may play a significant role in determining the amount of nitrogen this species fixes in soils with moderate mineral N available.

The amount of nitrogen fixed followed a pattern similar to the response to inoculation for common bean, but fertilizer N applied at planting suppressed nitrogen fixation by soybean. These results indicate that management recommendations for these two species must be considered individually in these low

N soils. Although inoculation of both species is imperative, the farmer would be better off allocating his production resources to inputs other than fertilizer nitrogen for soybean production; but, the purchase of small amounts of nitrogen for common bean would be an extremely wise investment. Efforts were made to relate this response to a measure of N concentration in an index tissue (most recently expanded trifoliate). These results are not yet complete but may provide a simple test that can be used early in crop growth to detect and ameliorate N deficiency.

Role of Phosphorus Management in the Response to Legume Inoculation With Rhizobia

If sufficient effective rhizobia are present either through inoculation or in a native population in the soil, management inputs that increase yield will also increase the need for the crop to assimilate nitrogen. In soils with moderate to high nitrogen which is common to many upland tropical soils of volcanic origin, management that increases yield may also enhance the use of soil N by the legume crop. An upland site in Bicol Province of the Philippines was used to examine how P management and inoculation interacted to affect yield, the response to inoculation, and the relative amounts of plant N derived from symbiotic and soil sources. The research was conducted in collaboration with Camarines Sur State Agricultural College (CSSAC). Soybean, common bean, peanut, and mungbean were grown under four levels of P management and either with or without inoculation. Corn was used as a reference species to measure nitrogen fixation by isotope dilution.

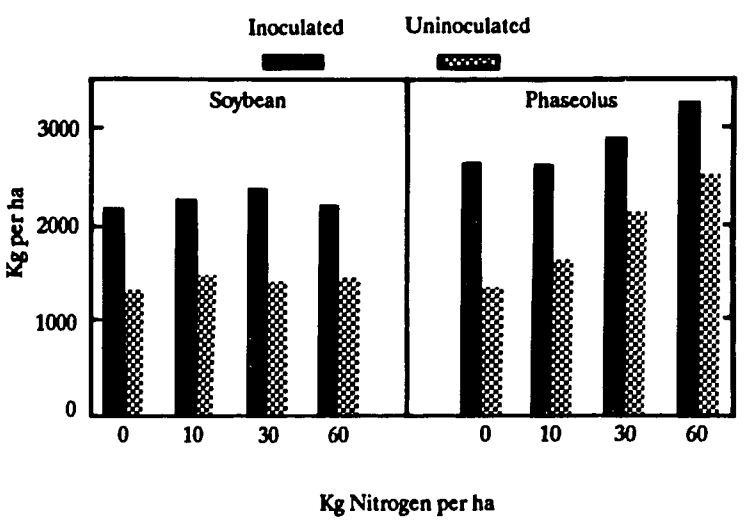
Soybean and mungbean responded significantly to inoculation and the response to inoculation increased as P inputs increased. For example, seed yield increases due to inoculation were 188 and 198 kg/ha for soybean and mungbean when there was no addition of P to the system. When 60 kg P/ha was applied, the increased yield from inoculation was 506 and 297 kg/ha for soybean and mungbean. The increase in yield of inoculated mungbean and soybean was more than 500 and 1200 kg/ha respectively when 60 kg P/ha was applied. These results demonstrate that there is a significant synergism between inoculation inputs and other management inputs for legume production systems. The farmer will obtain the greatest economic return to investment in each input only when both inputs are applied properly to the system. These experiments point to the danger of interpreting results of inoculation response trials without considering other factors that impact the system.

The Conservation of Fixed Nitrogen

Isotope enrichment data for all the species from both sites is not available yet, but results from mungbean and soybean at CSSAC indicated that P management played an important role in determining the amount of nitrogen fixed by legumes. Both the amount and proportion of plant nitrogen from fixation increased with additions of P fertilizer. The amount of N assimilated by the plants from soil sources also increased with better P nutrition. Results from soil analysis indicate that P did not affect soil N availability and the increase in plant uptake of soil N was related to plant growth. Although the overall N balance in this N rich environment was negative when legumes were grown and the harvest removed from the field, the balance was more favorable when P was applied to the system.

Seed Yield of Soybean and Phaseolus

Batac, Ilocos Norte, Philippines



Results from the low N environment (MMSU) indicate that the N balance of the species was generally positive but varied by species. Peanut, for example, fixed 95% of its total plant nitrogen at all levels of starter N applied compared to soybean (70-80%) and common bean which fixed between 20 and 50% of total N (see the figure below). Clearly, choice of species and management and the interaction with the environment are critical determinants in whether legume production will have a net benefit to soil N reserves. The notion that legumes enrich soil with nitrogen requires scrutiny and caution.

Appendices

Funds from Outside USAID Core Budget

Contracts and Grants Awarded Outside USAID Cooperative Agreement (last five years)

Grant Title	Source	Total
Soil Inoculation to Improve Crop Production	USAID/INDO-US	288,000
Host Control of Nodule Formation & Development	USDA/Competitive Grants	80,000
Predicting Inoculation Response	NSF/Ecology	750,000
Effect of Some Stress Factors on the Nodulation Process	USDA	23,000
Production & Conservation of Fixed N in Tropical Cropping Systems: Interaction Between Management & Environment	USDA/USAID Limiting Factors	87,000
Quantifying, Characterizing & Maximizing BNF by Fast-growing Nitrogen Fixing Trees	McIntire-Stennis/USAID	100,000
Workshop on "Dynamics of Soil Organic Matter in Tropical Ecosystems"	NSF/Ecology	80,000
Diversified Agriculture Research Project	USAID	16,000
Workshop on "BNF Technology Transfer"	USAID	80,000
Development of Acid-Soil Tolerant Leucaena Lines for Forage Production	USAID	151,000
Optimization of Rice-Based Systems in Negros Occidental	USDA/USAID Limiting Factors	73,000
BNF/Legume Management Outreach Pilot Project	USAID	107,000
Ecological and EvolutionaryAdaptation of Symbiotic Nitrogen Fixing Bacteria Associated with Native Hawaiian Leguminous Trees	The Nature Conservancy of Hawaii	8,000
Commercial Inoculum Production for Haiti	AID/Port-au-Prince	50,000

University of Hawaii Support of Core Operational Funding

Fiscal Year	85-86	86-87	87-88	88-89	89-90
Position 1 (Director)	21,250	85,000	85,000	85,000	85,000
Position 2 (Researcher)		70,000	70,000	70,000	70,000
Core Operations (Equip/ Materials/Supplies)		35,000	50,000	50,000	50,000
Returned Overhead	29,400	39,300	32,831	49,221	47,502
Repair & Maintenance			176,000	99,000	200,000
Vice-President Funds		50,000	50,000	50,000	
Equipment			69,000	37,000	10,000
UH Foundation			30,000		
Total	50,650	279,300	562,831	440,221	462,502

Abstracts of Published Work (1988-89)

Abaidoo, Robert C. and Christopher Van Kessel, 1989. ^{15}N-Uptake, N_2-Fixation and Rhizobial Interstrain Competition in Soybean and Bean, Intercropped with Maize. Soil Biol. Biochem. 21(1): 155-159.

N_2-fixing activity of soybean and beans monocropped or intercropped with maize was measured by the ^{15}N dilution method. Intercropped bean and soybean, planted in the same hole with maize or in two separate planting holes, showed a decrease in atom % ^{15}N as compared with monocropped bean and soybean. However, compared to when monocropped, intercropped non-nodulating soybean isoline and uninoculated bean showed a highly significant decrease in atom % ^{15}N. Whereas the total amount of ^{15}N-label accumulated in monocrop and intercrop systems remained constant, intercropped maize accumulated more and intercropped legumes less ^{15}N compared with monocropped maize or legume, respectively. The decrease in atom % ^{15}N excess of intercropped N_2-fixing legumes could be explained by higher N_2-fixing activity, a different ^{15}N-uptake pattern or a difference in competition for soil N between intercropped legumes and maize.

Intercropping bean or soybeans did not alter the competition pattern of the introduced strains of rhizobia. TAL 102 (USDA 110) and TAL 182 were the best competitors for soybeans and beans, respectively, and formed the greatest number of nodules, independent of cropping systems tested.

Bohlool, B. 1988. Rhizobium Technology: Application for International agricultural development in the tropics. In Bothe/de Bruijn/Newton (eds.) *Nitrogen Fixation: Hundred Years After*, Stuttgart, New York, pp. 759-764.

A nation's wealth and prosperity is intimately related to the quality of its soils. Is it any wonder, then, that most of the poorer nations of the world are located in the tropics where the vast majority of the soils are infertile and stressful? The impoverished state of the under-utilized soils of the tropics stems from their high degree of weathering. Soil minerals have been eroded to such an extent that they are no longer capable of holding essential plant nutrients. Many tropical soils are acid, high in aluminum or manganese and low in bases.

Among the plethora of adverse biotic and abiotic factors in the tropics, nitrogen (N) and phosphorus (P) can be singled out as two of the most important soil factors that limit agricultural production. Chemical fertilizers are costly, requiring large capital investment for production, storage, transportation and application. Developing countries (LDs) suffer not only from the high cost of fertilizers on the local market, but also from the lack of hard currency to play for them on the world market. Biological processes which control the delicate balance of both nitrogen and phosphorus in the soil can contribute to making N and P more readily available for crop plants. Technologies based on such processes, therefore, are of particular significance to agricultural development in the tropics.

George, T., P.W. Singleton, and B.B. Bohlool, 1988. Yield, soil nitrogen uptake, and nitrogen fixation by soybean from four maturity groups grown at three elevations. Agron J 80(4): 563- 567.

The exploitation of the soybean [*Glycine max* (L.) merr.] - *Bradyrhizobium japonicum* symbiosis in varied environments requires an understanding of factors that may affect fixed and soil N assimilation. Temperature affects both soybean maturity and N requirement, and soil N availability. Five soybean varieties belonging to four maturity groups (00, IV, VI, and VIII) and their respective non-nodulating isolines were planted at three sites along an elevational transect in Hawaii to study the effect of elevation-associated changes in temperature on yield, soil N uptake, and N_2 fixation. The mean soil/air temperatures during the experiment were 24/23, 23/21, and 20/18°C, with 7, 8, and 9°C differentials between mean maximum and mean minimum air temperatures at elevations of 320, 660, and 1050 m, respectively. The soils of the two lower sites were Humoxic Tropohumults and that of the highest site was Endic Dystrandept. The seeds were inoculated at planting with *B. japonicum*. Plants were harvested at physiological maturity. Average dry matter yield and N accumulation at the highest site was only 48 and 41%, respectively, of that at the lowest site. Early maturing varieties were relatively more affected by high elevation than were later varieties. Within each site, late-maturing varieties produced greater yield and accumulated more N than did the early varieties. Nitrogen fixation contributed 80, 66, and 97% of total plant N at the lowest, intermediate, and highest sites, respectively. Differences between sites in the proportion of N from fixation were due to differences in the availability and uptake of soil N. Although total N assimilation between varieties differed in some cases by more than 400%, the proportions of N derived from fixation were similar within a site. On a per-day basis, N accumulation was similar among varieties at a site. Since the different maturity groups had similar average N assimilation rates per day within a site, and since soil N mineralization rate per day at a site can be assumed to

be constant, the proportion of total N derived from N_2 fixation is necessarily constant. Since the proportion of N derived from fixation is yield independent at a site, it may be possible to predict the relative contributions of soil N and symbiotic N_2 fixation to soybean through characterization of the processes that affect N availability at a site. Data from in vitro soil N mineralization and a greenhouse pot test indicated that low temperature may have decreased root growth and uptake of soil N, or soil N availability more than it reduced N_2 fixation.

Olsson, Jane E., Patricia Nakao, B. Ben Bohlool, and Peter M. Gresshoff, 1989. Lack of Systemic Supression of Nodulation in Split Root Systems of Supernodulating Soybean (*Glycine max* [L.] Merr.) Mutants. Plant Physiol. 90: 1347-1352.

Wild-type soybean (*Glycine max* [L] Merr. cv Bragg) and a nitrate-tolerant supernodulating mutant (nts382) were grown in split root systems to investigate the involvement of the autoregulation response and the effect of timing of inoculation on nodule suppression. In Bragg, nodulation of the root portion receiving the delayed inoculation was suppressed nearly 100% by a 7-day prior inoculation of the other root portion with *Bradyrhizobium japonicum* strain USDA 110. Significant suppression was also observed after a 24-hour delay in inoculation. Mutant nts382 in the presence of a low nitrate level (0.5 millimolar) showed little, if any, systemic suppression. Root fresh weights of individual root portions were similar for both wild type and nts382 mutant. When nts382 was grown in the absence of nitrate, a 7-day delay in inoculation resulted in only 30% suppression of nodulation and a significant difference in root fresh weight between the two sides, with the delayed inoculated side always being smaller. Nodulation tests on split roots of nts382, nts 1116, and wild-type cultivars Bragg, Williams 82, and Clark demonstrated a difference in their systemic suppression ability. These observations indicate that (a) autoregulation deficiencies in mutant nts382 result in a reduction of systemic suppression of nodulation, (b) some suppression is detectable after 24 hours with a delayed inoculation, (c) the presence of low nitrate affects the degree of suppression and the root growth, and (d) soybean genotypes differ in their ability to express this systemic suppression.

Rangarajan, M., P. Somasegaran, H. Hoben, 1988. Serological relatedness among strains of Rhizobium meliloti originated from different geographical regions. National Academy Sci. Ltrs. 11(10): 303-305.

Serological analysis of 9 effective strains out of 26 strains of *Rhizobium meliloti* from different parts of Maui island of Hawaii along with the strain TAL 380 from Australia and TAL 1171 of Wisconsin of continental U.S.A., revealed the antigenic similarity among them. The results of the somatic cross agglutination reactions of eleven strains indicated that six strains from Hawaii (TAL 1477, 1481, 1483, 1496, 1499, and 1502) were serologically related to TAL 380 of Australia while two more strains (TAL 1488 and TAL 1501) of the former place were serologically related to TAL 1171 of Wisconsin.

Romero, D., P.W. Singleton, et al., 1988. Effect of naturally occurring nif reiterations on symbiotic effectiveness in *Rhizobium phaseoli*. Appl. Env. Micro. 54(3): 848-850.

Most naturally occurring strains of *Rhizobium phaseoli* possess reiteration of the *nif* genes. Three regions contain nitrogenase structural genes in strain CFN42. Two of these regions (a and b) have copies of *nifH*, *nifD*, and *nifK*, whereas the third region (c) contain only *nifH*. Strains containing mutations in either *nif* region a or *nif* region b had significantly diminished symbiotic effectiveness compared with the wild-type strain on the basis of nodule mass, total nitrogenase activity per plant, nitrogenase specific activity, total nitrogen in shoot, and percentage of nitrogen. A strain containing mutations in both *nif* region a and *nif* region b was totally ineffective. These data indicate that both *nif* region a and *nif* region b are needed for full symbiotic effectiveness in *R. phaseoli*.

Somasegaran, P., H. J. Hoben, and Velittin Gurgun, 1988. Effect of Inoculation Rate, Rhizobial Strain Competition, and Nitrogen Fixation in Chickpea. Agronomy J. 80: 68-73.

Chickpea (*Cicer arietinum* L.) is the third most widely cultivated legume in the world, but N-fixation studies addressing its inoculation response and rhizobial strain competition are limited. We investigated N fixation and competition by three strains of chickpea *Rhizobium* (TAL numbers 480, 620, and 1148) on 'Desi' and 'Kabuli' chickpea genotypes grown in vermiculite and two soils (Ultisol and Oxisol) free of chickpea rhizobia. Chickpea grown in vermiculite was inoculated with single and mixed strains. A three-strain mixture was inoculated at 10, 10^2, 10^4, 10^6, and 10^8 rhizobia per seed on seeds planted in the two soils. Nodulation and N-fixing parameters were significantly different for chickpea genotypes and rhizobial strains, with no genotype x strain interaction in the vermiculite experiment. TAL 620 was the most competitive strain in both chickpea genotypes. Maximum N fixation in both genotypes was achieved at 10^4 and 10^6 rhizobia per seed in the Ultisol and Oxisol, respectively. Nitrogen fixation by chickpea in response to different inoculation rates was best described by a quadratic model. The three strains of *Rhizobium* competed differently in the two soils. Competition in the Ultisol was due to significant strain differences and genotype x strain interaction. In the Oxisol, there was no stain competition. Different inoculation rates did not influence strain competition. In general, the Kabuli genotype had a greater

N-fixing potential than the Desi. Maximum N fixation in the two soils was a function of the different inoculation rates for both genotypes. Competition among chickpea rhizobia was influenced by the growth medium, chickpea genotypes, and strain differences but not inoculation rates.

van Kessel, C. and J.P. Roskoski, 1988. Row spacing effects on N_2 fixation, N-yield and soil N uptake of intercropped cowpea and maize. Plant and Soil 111: 17-23.

In the tropics, cowpea is often intercropped with maize. Little is known about the effect of the intercropped maize on N_2-fixation by cowpea or how intercropping affects nitrogen fertilizer use efficiency or soil N-uptake of both crops. Cowpea and maize were grown as a monocrop at row spacings of 40, 50, 60, 80, and 120 cm and intercropped at row spacing of 40, 50, and 60 cm. Plots were fertilized with 50 kg N as $(NH_4)_2SO_4$; microplots within each plot received the same amount of ^{15}N-depleted $(NH_4)_2SO_4$. Using the ^{15}N-dilution method, the percentage of N derived from N_2-fixation by cowpea and the recovery of N- fertilizer and soil N-uptake was measured for both crops at 50 and 80 days after planting.

Significant differences in yield and total N for cowpea and maize at both harvest periods were dependent on row spacing and cropping systems. Maize grown at the closer row spacing accumulated most of its N during the first 50 days after planting, whereas maize grown at the widest row spacing accumulated a significant portion of its N during the last 30 days before the final harvest, 80 days after planting. Overall, no significant differences in the percentage of N derived from N_2-fixation for monocropped or intercropped cowpea was observed and between 30 and 50% of its N was derived from N_2.

At 50 DAP, fertilizer and soil N uptake was dependent on row spacing with maize grown at the narrowest row spacing having a higher fertilizer and soil N recovery than maize grown at wider spacings. At 50 and 80 DAP, intercropped maize/cowpea did not have a higher fertilizer and soil N uptake than monocropped cowpea or maize at the same row spacing. Monocropped maize and cowpea at the same row spacing took up about the same amount of fertilizer or soil N. When intercropped, maize took up twice as much soil and fertilizer N as cowpea. Apparently intercropped cowpea was not able to maintain its yield potential.

Whereas significant differences in total N for maize was observed at 50 and 80 DAP, no significant differences in the atom % ^{14}N excess were observed. Therefore, in this study, the atom % ^{14}N excess of the reference crop was yield independent. Furthermore, the similarity in the atom % ^{14}N excess for intercropped and monocropped maize indicated that transfer of N from the legume to the non-legume was small or not detectable.

van Kessel, C., J.P. Roskoski and K. Keane, 1988. Ureide production by N_2-fixing and non-N_2-fixing leguminous trees. Soil Bio. Biochem. 20(6) 891-897.

Xylem-sap and stem and leaf extracts from 35 species, comprising 14 genera, of leguminous trees were analyzed for ureides, nitrate and –amino acids. Trees were either inoculated with *Rhizobium* or fertilized with NH_4NO_3. The dominant form of soluble N in stem and leaf extracts and xylem sap was –amino acids. Certain non- N_2-fixing species, i.e., *Tamarindus indica* and *Adenanthera pavonina*, produced significant amounts of ureides. Several N_2- fixing species, *Mimosa scabrella*, *Sesbania grandiflora*, *Acacia meamsii* and *Gliricidia sepium*, grown on mineral-N had higher absolute amounts of ureides in both extracts and exudates than did most nodulated species. Nodulated *A. meamsii* and *S. grandiflora*, had the highest amounts of ureides in xylem sap. The relative abundance of ureides n stem and leaf extracts was lower than in xylem sap, but was correlated. Results indicated that the presence of ureides, per se, was not a reliable indicator of N_2-fixing activity. Moreover, the relative abundance of ureides in most of the species tested was too low to use as a presumptive test for, or as a means of estimated N_2 fixation.

Woomer, Paul, P.W. Singleton, and B.Ben Bohlool, 1988. Ecological indicators of native rhizobia in tropical soils. Appl. Env. Microbiol. 54(5): 1112-1116.

The relationship between environment and abundance of rhizobia was described by determining the populations of root nodule bacteria at 14 diverse sites on the island of Maui. Mean annual rainfall at the sites ranged from 320 to 1,875 mm, elevation from 37 to 1,650 m, and soil pH form 4.6 to 7.9. Four different soil orders were represented in this study: inceptisols, mollisols, ultisols, and an oxisol. The rhizobial populations were determined by plant infection counts of five legumes (*Trifolium repens*, *Medicago sativa*, *Vicia sativa*, *Leucaena leucocephala*, and *Macroptilium atropurpureum*). Populations varied from 1.1 to 4.8 log10 cells per g of soil. the most frequently occurring rhizobia were *Bradyrhizobium* spp., which were present at 13 of 14 sites with a maximum of 4.8 log 10 cells per g of soil. *Rhizobium trifolii* and *R. leguminosarum* occurred only at higher elevations. The presence of a particular *Rhizobium* or *Bradyrhizobium* sp. was correlated with the occurrence of its appropriate host legume. Total rhizobial populations were significantly correlated with mean annual rainfall, legume cover and shoot biomass, soil temperature, soil pH, and phosphorus retention. Regression models are presented which describe the relationship of legume hosts, soil climate, and soil fertility on native rhizobial populations.

Woomer, Paul L., Paul W. Singleton, and B. Ben Bohlool, 1988. Reliability of the Most-Probable-Number technique for enumerating rhizobia in tropical soils. Appl. Envir Microbiol. 56(6): 1494-1497.

We used six rhizobium-legume systems to test the reliability of the most-probable-number (MPN) technique for enumerating rhizobia introduced into 14 sites representing four soil orders. The range-of-transition values (the number of dilution steps between the first not-entirely-positive and the last not- entirely-negative growth units) were compared for each species and for each soil. the probability that the observed data were significantly different from theoretical values varied with the species. The acceptability of MPN codes (P. 0.99) was the highest (97 to 99%) with *Vicia sativa*, *Trifolium repens*, and *Glycine max* and lowest (72%) with *Leucaena leucocephala*. *Medicago sativa* and *Macroptilium atropurpureum* yielded 87 and 75% acceptable MPN codes, respectively. The acceptability of the MPN data obtained for a host species was related to rooting habit and time to nodulation. Comparison of data for each soil indicated that, despite large differences in characteristics, the soil was not a major source of variability in the MPN counts. There was no significant interaction of the range of transition of rhizobium-legume plant infection count data between species and site.

Woomer, Paul and B.Ben Bohlool, 1989. Rhizobial Ecology in Tropical Pasture Systems. In. Marten et al (Eds), *Persistence of Forage Legumes*. ASA, Madison, WS: 233-245.

We have studied the ecology of rhizobia in the diverse climates and vegetation of Maui, Hawaiian Islands. Many of the sites represent predominant tropical pasture systems including short and tall grass savannas, and upland and lowland grassy pastures. The population size of the rhizobia in the soils and rhizospheres of these pastures is correlated with environmental factors. The importance of the legume component on rhizobial populations is established. The frequency of observing both associated legumes and rhizobia at a site is .94. The density of legumes correlates significantly with the density of rhizobia in soils. Conceptual and regression models are presented which predict the population size of rhizobia in tropical pasture system and which describe the environmental influences of native and introduced rhizobia in the soils supporting host and non-host swards. Also presented is an update of the new rhizobial taxonomic system relevant to the interest of pasture scientists.

Bibliography 1976-1989

Abaidoo, R. and C. vanKessel, 1989. ^{15}N Uptake, N_2 fixation and *Rhizobium* interaction in soybean and bean intercropped with maize. Soil Biol. Biochem 21:155-159.

Akyeampong, M., 1980. The Effect of Different Strains of *Rhizobium* and Phosphorus Levels in Mungbean (*Vigna radiata*) and Soybean (*Glycine max*). M.S. Thesis, Univ of Hi., 56 pp.

Ayanaba, A., Asanuma, S., and D.N. Munns, 1981. An Agar Plate Method for Rapid Screening of *Rhizobium* for tolerance to Acid-Aluminum Stress. Research Briefs 2(4):5-8.

Ayanaba, A., Asanuma, S., and D.N. Munns, 1983. An Agar Plate Method for Rapid Screening of *Rhizobium* for Tolerance to Acid-Aluminum Stress. Soil Sci. Soc. Am. J. 47:256-258.

Aziz, T. and M. Habte, 1984. Effect of Mycorrhizal Fungi on the Growth of *Sesbania grandiflora*. Agron. Abst., pp. 12.

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

Beck, D.P., B. Lertbhuddikhul, 1985. BNFRC Newsletter for South and Southeast Asia. Vol 2, July 1985, 8 pp.

Beck, D.P., D.N. Munns, 1985. Effect of Calcium on the Phosphorus Nutrition of *Rhizobium meliloti*. Soil Sci. Soc. Am. J. 49: 334-337.

Berger, J.A., May, S.N., Berger, L.R., and B.B. Bohlool, 1979. Colorimetric Enzyme-Linked Immunosorbent Assay for the Identification of Strains of *Rhizobium* in Culture and in the Nodules of Lentils. Appl. Envir. Microbiol. 37(3):642-646.

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.

Bohlool, B.B. 1987. Fluorescence Methods for Study of *Rhizobium* culture and soils. In. G.H. Elkan (ed) Symbiotic Nitrogen Fixation Technology, Marcel Dekker Inc., New York, p. 127-147

Bohlool, B. 1988. Rhizobium technology: Application for International agricultural development in the tropics. In Bothe/de Bruijn/Newton (eds) *Nitrogen Fixation: Hundred Years After*, Stuttgart, New York, p. 759-764.

Bose, John II, 1978. The Legume/*Rhizobium* Symbiosis in Tropical Agriculture: A Selective Bibliography with Annotations. NifTAL Project, 193 pp.

Bose, John II, 1980. The Legume/*Rhizobium* Symbiosis in Tropical Agriculture: A Bibliographic Update. NifTAL Project, Hawaii, 167 pp.

Burton, J.C., 1981. *Rhizobium* Inoculants for Developing Countries. Trop. Agric. (Trinidad) 58(4):291-295.

Burton, J.C., 1982. Modern Concepts in Legume Inoculation. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

Burton, J.C., 1983. *Legume Inoculants and Their Use*. FAO, Rome, 85 pp.

Burton, J.C., 1983. TDY Report - Legume Inoculant (Rhizobia) Production for Soybeans and Other Legumes in Egypt. EMCIP Publication No. 5, Consortium for Int'l. Development, Publishers, 30 pp.

Burton, J.C., 1984 *Legume Inoculant Production*. NifTAL, Hawaii, 50 pp.

Burton, J.C., 1985. *Rhizobium* Relationships. In Clover Science and Technology, Agronomy Monog. #25: 161-184.

Cassmann, K.G., 1979. The Phosphorus Nutrition of Two Grain Legumes as Affected by Mode of Nitrogen Nutrition. Ph.D. Thesis, Univ. of Hi., 95 pp.

Cassman, K.G., Munns, D.N., and D.P. Beck, 1981. Phosphorus Nutrition of *Rhizobium japonicum*: Strain Differences in Phosphate Storage and Utilization. Soil Sci. Soc. of Amer. J. 45(3):517-520.

Cassman, K.G., Munns, D.N., and D.P. Beck, 1981. Growth of *Rhizobium* Strains at Low Concentrations of Phosphate. Soil Sci. Soc. of Amer. J. 45(3):520-523.

Cassman, K.G., Whitney, A.S., and R.L. Fox. 1981. Phosphorus Requirements of Soybean and Cowpea as Affected by Mode of N Nutrition. Agron J 73:17-22.

Cassman, K.G., Whitney, A.S., and R.L. Fox, 1980. Symbiotic Nitrogen Fixation by Legumes Requires Extra Phosphorus. Illustrated Concepts in Tropical Agriculture, Univ. of Hi., Coll. Trop. Ag. Hum. Res., 1 pp.

Cassman, K.G., Whitney, A.S., and K.R. Stockinger, 1980. Root Growth and Dry Matter Distribution of Soybean as Affected by Phosphorus Stress, Nodulation, and Nitrogen Source. Crop Sci. 20(2): 239-244.

Cunningham, Scott D., and D.N. Munns, 1984. Effects of Rhizobial Extracellular Polysaccharide on pH and Aluminum Activity. Soil Sci. Soc. Am. J. 48:1276-1280.

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

DaSilva, E.J., Freire, J.R.J., Keya, S.O., and J. Halliday, 1983. The Role of MIRCENS in Rural Development, Agricultural Training and Environmental Management. In Training for Agriculture and Rural Development 1982, FAO, p. 23-28.

Davis, R.J., 1981. Viability of Lyophilized Cultures Stored for 37 Years. In K.W. Clark and J.N.G. Stephens (Eds.) Proceedings 8th North Amer. Rhizobium Conference, Winnipeg, Manitoba, p. 372-375.

Davis, R.J., Halliday, J., and P. Somasegaran, 1981. A Worldwide Network of Inoculation Trials. In A.H. Gibson and W.E. Newton (Eds.) Current Perspectives in Nitrogen Fixation. Australian Acad. of Sci., p. 515.

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.

Davis, R.J, Cady, F.B., Wood, C.L., and Chan, C.P.Y. 1985. Design and Analysis of an International Experimental Network: Legume Inoculation Trials in the NifTAL Project, the INLIT Experience. U HI Research Series 042, 43 pp.

Davis, R.J. 1985. Legume Inoculation. Bangladesh Agricultural Research Council.

Dowdle, S.F., 1984. China's Technical Contract System: Linking Agricultural Technology to Economic Incentives. Agron. Abst. p. 40.

Dowdle, S.F. and B.B. Bohlool, 1984. Predominance of Indigenous Fast-Growing *Rhizobium japonicum* in a Soybean Field in the People's Republic of China. Agron. Abst. p. 185.

Dowdle, S.F., B.B. Bohlool, 1985. Predominance of Fast-growing *Rhizobium japonicum* in a Soybean Field in the People's Republic of China. Appl. Env. Micro. 50(5): 1171-1176.

Dowdle, S.F. and B.B. Bohlool, 1987. Intra- and Inter-Specific Competition in *Rhizobium fredii* and *Bradyrhizobium japonicum* as indigenous and introduced organisms. Can. J. Microbiol. 33: 990-995.

Eriksen, F.I., and A.S. Whitney, 1981. Effects of Light Intensity on Growth of Some Tropical Forage Species. I. Interaction of Light Intensity and Nitrogen Fertilization on Six Forage Grasses. Agron. J. 73:427-433.

Eriksen F.I., and A.S. Whitney, 1982. Growth and N Fixation of Some Tropical Forage Legumes as Influenced by Solar Radiation Regimes. Agron. J. 74:703-709.

Eriksen, F.I., and A.S. Whitney, 1984. Effects of Solar Radiation Regimes on Growth and N_2 fixation of Soybean, Cowpea, and Bushbean. Agron. J. Vol. 76(4):529-535.

Fox, R.L., and A.S. Whitney, 1981. Response of Leucaena leucocephala to Lime Applications in Hawaii. Leucaena Res. Reports 2:69-70.

George, T., B.B. Bohlool, and P. Singleton, 1987. *Bradyrhizobium japonicum*-Environment Interactions: Nodulation and Interstrain Competition in Soils along an Elevational Transect. Appl. Env. Microbiol. 53(5):113-117.

George, T., P.W. Singleton, and B.B. Bohlool, 1988. Yield, soil nitrogen uptake, and nitrogen fixation by soybean from four maturity groups grown at three elevations. Agron J 80(4): 563-567.

Graham, P.H., Apolitano, C., Ferrera-Cerrato, R., Halliday, J., Lepiz, R., Menendez, O., Rios, R., Saito, S.M.T., and S. Viteri, 1982. The International Bean Inoculation Trial (IBIT): Results for the 1978-1979 Trial. BNF Tech. for Tropical Agric. p. 223-229.

Graham , P.H., and S.C. Harris. 1983. (Eds.) Biological Nitrogen Fixation Technology forTropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de AgriculturaTropical, 768 pp.

Gueverra, A.B., B., Kitamura, Y., Whitney, A.S., and K.G. Cassman, 1980. A Low-Cost System for Circulating Nutrient Solutions in Pot Studies. Crop Sci. 20(1):110-112.

Guevarra, A.B., Whitney, A.S., and J.R. Thompson, 1978. Influence of Intra-Row Spacing and Cutting Regimes on the Growth and Yield of Leucaena. Agron. J. 70:1033-1037.

Habte, Mitiku, 1985. Selective Medium for vering Specific Populations of Rhizobia Introduced into Tropical Soils. Appl. Env. Micro. 50(6): 1553-1555.

Halliday, J., 1981. Biological Nitrogen Fixation: Its Potential in Tropical Soils. In S.O. Emejuaiwe, O. Ogunbi and S.O. Sanni (Eds.) Global Impacts of Applied Microbiology. Academic Press, London, p. 73-84.

Halliday, J., 1981. Paper No. 5, Agrotechnologies Based on Symbiotic Systems That Fix Nitrogen. An Office of Technology Assessment Workshop, Background Papers for Innovative Biological Technologies for Lesser Developed Countries. Report Prepared for Committee on Foreign Affairs, U.S. House of Representatives. p. 243-273.

Halliday, J., 1981. Nitrogen Fixation by Leucaena in Acid Soils. Leucaena Res. Reports 2:71-72.

Halliday, J., 1982. La Fijacion Biologica de Nitrogeno como Una Agrotechnolgia Transferible en el Tropico. In, Ayala, L.B. (ed.), Aspectos Biologicos Agronomicos y Technologicos de la rhizobiologia. FONAIAP (Venezuela) Serie Especial No. 2-02: 25-35.

Halliday, J. 1982. Present Constraints to, and a Future Strategy For, Fuller Utilization of Legume-Fixed Nitrogen for Crop Production in Tropics. Food and Fert. Tech. Center Tech. Bull. 60:1-23.

Halliday, J., 1983. Rhizobium Relations of Tropical Legumes in Tropical Soils andImplications for Agrotechnology Transfer. Benchmark Soils Project Tech. Note No. 7: 129-140.

Halliday, J. 1983. Biological Nitrogen Fixation: What's In It For Hawaii? In Proceedings 1st Fertilizer and Ornamentals Workshop, Univ. of Hi. Research Extension Series 037: 75-86.

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

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.

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.

Halliday, J., 1984. Principles of *Rhizobium* Strain Selection. In M. Alexander, (Ed.) Biological Nitrogen Fixation. Plenum Publ, Corp. p. 155-171.

Halliday, J., 1984. Guidelines for Collecting the Root Nodules of Leguminous Trees. Nitrogen Fixing Tree Res. Reports Vol. 2.

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.

Halliday, J., King, J.W., and P. Nakao, 1982. Biological Nitrogen for Pastures. Proceedings: 17th Mealani Beef Cattle Field Day, Mealani Expt. Stn., Kamuela, Hawaii.

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

Halliday, J., Nakao, P., and J.W. King, 1982. Practical Steps for Seed Preparation of Pasture Legumes. Proceedings: 17th Mealani Beef Cattle Field Day, Mealani Expt. Stn., Kamuela, Hawaii.

Halliday, J., and P. Somasegaran, 1983. Nodulation, Nitrogen Fixation, and *Rhizobium* Strain Affinities in the Genus Leucaena. Leucaena Research in the Asian-Pacific Region. IDRC, p. 27-32.

Halliday, J., and P. Somasegaran, 1984. The Rhizobium Germplasm Resource and NifTAL Catalogue of Strains. NifTAL Project, 94 pp.

Harris, S.C. (Ed.), 1979. Planning an International Network of Legume Inoculation Trials: The Proceedings of a Workshop held at Kahului, Maui, Hawaii. NifTAL Project, 241 pp.

Harris, S.C., 1982. Internationally Sponsored Development of Biological Nitrogen Fixation Technology. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

Hoben, H.J., and P. Somasegaran, 1982. Comparison of the Pour, Spread, and Drop Plate Methods for Enumeration of *Rhizobium* spp. in Inoculants Made from Presterilized Peat. Appl. Environ. Microbiol. 44(5):1246-1247.

Hoben, H. and P. Somasegaran 1985. NifTAL Rhizobium Antisera Resource Catalogue. NifTAL 14 pp.

Hoben, H. and P. Somasegaran. 1986. A Simple-Transfer Chamber for Aseptic Work with Microorganisms. NifTAL Illustrated Concepts in Agricultural Biotechnology Series No. 1, 1986.

Hoben, H.J. and P. Somasegaran, 1987. A Small Glass Fermenter for The Production of *Rhizobium* Inoculum. NifTAL Illustrated Concepts in Agricultural Biotechnology Series No. 2, 1/1987.

Hohenberg, J.S., Munns, D.N., and C.L. Tucker. 1982. *Rhizobium*-Host Specificities in *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. Crop Sci. 22(3):455-459.

Keyser, H.H., and D.N. Munns, 1979. Effects of Calcium, Magnanese, and Aluminum on Growth of Rhizobia in Acid Media. Soil Sci. Soc. of Amer. J. 43(3):500-503.

Keyser, H.H., and D.N. Munns, 1979. Tolerance of Rhizobia to Acidity, Aluminum, and Phosphate. Soil Sci. Soc. Amer. J. 43(3):519-523.

Keyser, H.H., Munns, D.N., and J.S. Hohenberg,1979. Acid Tolerance of Rhizobia in Culture and in Symbiosis with Cowpea. Soil Sci. Soc. Amer. J. 43(4):719-722.

Keyser, H.H., M.J. Sadowsky, and B.B. Bohlool, 1985. Fast-Growing Soybean Rhizobia. In World Soybean Research Conference III: Proceedings (Ed Richard Shibles), Westview Press. p. 926-934.

Kilkenny, M.R., and D.E. Welsch, 1982. An Economic Analysis of Quantitative Targets for BNF. Agron. Abstr. p. 212.

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

King, J.W., 1983. Pretesting Commumication Materials in International Agricultural Settings. Agric. Information Dev. Bull. 5(4):13-17.

King, J.W., 1983. Revising Training Media. Performance and Instruction J. 22(4):9-10.

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

Kitamura, Y., Whitney, A.S., and A.B. Guevarra, 1981. Legume Growth and Nitrogen Fixation as Affected by Plant Competition for Light and Soil Nitrogen. Agron. J. 73:395-398.

Kosslak, R.M. and B.B. Bohlool. Influence of Environmental Factors on Interstrain Competition in *Rhizobium japonicum*, 1985. Appl. Env. Micro. 49(5): 1128-1133.

Lumpkin, T.A., 1982. Chinese Technology for the Cultivation of Azolla. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen FixationTechnology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, CIAT, 768 pp.

Lumpkin, T.A., Zhuo-sing, Li, Shou-xian, Zu, and Mao Mei-fei, 1982. The Effect of Species of Azolla Under Three Management Practices on the Yield of Paddy Rice. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

May, S.N., 1979. Ecological Studies on Lentil Rhizobia: Competition and Persistence in Some Tropical Soils. M.S. Thesis, Univ of Hi., 54 pp.

May, S.N., and B.B. Bohlool, 1983. Competition Among *Rhizobium leguminosarum* Strains for Nodulation of Lentils (*Lens esculenta*). Appl. Environ. Microbiol. 45(3):960-965.

McNeil, D.L., 1982. Quantification of Symbiotic Nitrogen Fixation Using Ureides: A Review. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

McNeil, D.L. 1982. Variations in Ability of *Rhizobium japonicum* Strains to Nodulate Soybeans and Maintain Fixation in the Presence of Nitrate. Appl. Environ. Microbiol. 44(3):647-652.

McNeil, D.L., Borton, S., Amara, D., and M.S. Vora, 1983. Use of Antibiotic-Resistant *Rhizobium* Mutants for Competition Studies with *Cajanus cajan*. Int'l. Pigeonpea Newsltr. 2:71-72.

McNeil, D.L., Croft, L.K., and T.S. Sandhu. 1981. Response of Chickpeas to Inoculation with *Rhizobium* in Hawaii. Int'l. Chickpea Newsltr. 4:25-26.

Mirza, N.A., B.B. Bohlool, and P. Somasegaran, 1989. Non-destructive Chlorophyll Assay for Screening of Strains of *Bradyrhizobium japonicum*. Soil Biol. Biochem. (In Press)

Munns, D.N., et al. 1979. Tolerance of Soil Acidity in Symbiosis of Mung Bean with Rhizobia. Agron. J. 71(2):256-260.

Munns, D.N., and H.H. Keyser, 1981. Response of *Rhizobium* Strains to Acid and Aluminum Stress. Soil Biol. Biochem. 13:115-118.

Munns, D.N., and A.A. Franco, 1982. Soil Constraints to Legume Production. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

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

Olsson, J.E., P. Nakao, B.B. Bohlool, and P. M. Gresshoff, 1989. Lack of Systemic Suppression of Nodulation in Split Root Systems of Supernodulating Soybean (*Glycine max* [L.] Merr.) Mutants. Plant Physiol 90: 1347-1352.

Rangarajan, M., Somasegaran, P., and H.J. Hoben, 1983. Occurrence of Different Serotypes of *Rhizobium meliloti* in Hawaiian Soils. 6th Southern Regional Conference on Microbial Inoculants, Bangalore, India.

Rangarajan, M., Somasegaran, P., and H.J. Hoben, 1983. Variation in the Fluorescence of Bacteroids of *Rhizobium japonicum* and *Rhizobium* sp. (*Vigna unguiculata*) from Nodules of Homologous and Heterologous Hosts. 9th North American Rhizobium Conference, Ithaca, New York.

Rangarajan, M., Somasegaran, P., Hoben, H.J., and J. Halliday, 1983. Nodule Occupancy by Different Strains of a Multistrain Rhizobial Inoculant in Two Leucaena Cultivars. 6th Southern Regional Conference on Microbial Inoculants, Bangalore, India.

Rangarajan, M., P. Somasegaran and H. Hoben, 1988. Serological relatedness among strains of *Rhizobium meliloti* originated from different geographical regions. National Academy Sci. Ltrs. 11(10): 303-305.

Reyes, V.G., Somasegaran, P., and D.N. Munns, 1978. Catalog of Selected Strains from the NifTAL Rhizobium Collection. NifTAL Project, 16 pp. Symbiosis. Crop Sci. 23:259-262.

Rightti, R.L., and D.N. Munns, 1980. Nodulation and Nitrogen Fixation in Cliffrose (*Cowania mexicana*). Plant Physiol. 65:411.

Romero, D., P.W. Singleton, et al., 1988. Effect of naturally occurring *nif* reiterations on symbiotic effectiveness in *Rhizobium phaseoli*. Appl Env Micro 54(3): 848-850.

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.

Roskoski, J.P. and C. van Kessel, 1985. Annual, Seasonal and Diel variation in nitrogen fixing activity by *Inga jinicuil*, a tropical leguminous tree. Oikos 44: 306-312.

Roskoski, J.P., I. Pepper, and E. Pardo, 1986. Inoculation of Leguminous Trees with Rhizobia and Va Mycorrhizal Fungi. Forest Ecol. Management 16: 57-68.

Sadowsky, M.J. and B.B. Bohlool, 1985. Differential Expression of the Pea Symbiotic Plasmid pJB5JI in Genetically Dissimilar Backgrounds. Symbiosis 1: 125-138.

Sadowsky, M.J. and B.B. Bohlool, 1986. Growth of Fast- and Slow-growing Rhizobia on Ethanol. Appl. Env. Micro. 52(4): 951-952.

Singleton, P.W. 1979. Effect of Inoculation with *Rhizobium* bacteria on *Arachis hypogaea*. M.S. Thesis, Univ. of Hawaii, 70 pp.

Singleton, P.W., 1982. The Effect of Salinity on Rhizobium Survival, Nodule Function and Nodule Formation in the Soybean-*Rhizobium japonicum* Symbiosis. Ph.D. Thesis, Univ. of Hawaii, 82 pp.

Singleton, P.W., El Swaify, S.A, and B.B. Bohlool, 1982. Effect of Salinity on *Rhizobium* Growth and Survival. Appl. Environ. Microbiol. 44(4):884-890.

Singleton, P.W., and K.R. Stockinger, 1983. Compensation Against Ineffective Nodulation in Soybean. Crop Sci. 23:69-72.

Singleton, P.W., 1983. A Split-Root Growth System for Evaluating the Effect of Salinity on Components of the Soybean *Rhizobium japonicum* symbiosis. Crop Sci 23:259-262.

Singleton, P.W., and B.B. Bohlool, 1983. Effect of Salinity on the Functional Components of theSoybean-Rhizobium japonicum Symbiosis. Crop Sci. 23: 815-818.

Singleton, P.W., and B.B. Bohlool, 1983. The Effect of Salinity on Nodule Formation by Soybean. 9th North American Rhizobium Conference, Ithaca, New York.

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

Singleton, P., and B.B. Bohlool, 1984. The Effect of Salinity on Survival of *Rhizobium*, 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.

Singleton, P.W. and H.M. Abdel Magid, 1984. The Interaction Between P-defined Yield Potential and Effectiveness of Strains of *Rhizobium japonicum*. Proc. 7th Aust. Legume Nodulation Conference. Sydney, Australia.

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 Research Conference III Abst. Ames, Iowa.

Singleton, P.W. and J.W. Tavares, 1984. Response to Legume Inoculation as Affected by Native Rhizobial Populations. Agron. Abst. p. 193.

Singleton, P.W., Abdel Majid, H.M., and Tavares, J.W., 1985. Effect of Phosphorus on the Effectiveness of Strains of *Rhizobium japonicum*. Soil Sci. Soc. of Am. J. 49(3):613-616.

Singleton, P., J. Burton F. Cady, R. Davis, and J. Halliday. 1985. Developing Cost-Effective Rhizobium Technology for the Tropics and Sub tropics. In. Soybean In Tropical And Subtropical Cropping Systems. S. Shanmugasundaram and E. Sulzberger (ed.) Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Singleton, P. and C. vanKessel, 1987. Effect of Localized Nitrogen Availability to Soybean Half-Root Systems on Photosynthate Partitioning to Roots and Nodules. Plant Physiol. 83:552-556.

Somasegaran, P., Hoben, H., and J. Halliday, 1980. Practical Exercises in Legume/Rhizobium Technology. Univ. of Hawaii NifTAL Project.

Somasegaran, P. and J. Halliday, 1982. Dilution of Liquid *Rhizobium* Cultures to Increase Production Capacity of Inoculant Plants. Appl. Environ. Microbiol. 44(2):330-333.

Somasegaran, P., 1983. Two Methods for Inoculant Production with Diluted Liquid Cultures of Rhizobium and Autoclaved Peat. 9th North American Rhizobium Conference, Ithaca, New York.

Somasegaran, P., Reyes, V.G., and H.J. Hoben, 1983. The Influence of High Temperatures on the Growth and Survival of *Rhizobium* spp. in Peat Inoculants During Preparation, Storage and Distribution. 9th North American Rhizobium Conference, Ithaca, New York.

Somasegaran, P., Woolfenden, R., and J. Halliday 1983. Suitability of Oven-dried Root Nodules for *Rhizobium* Strain Identification by Immunofluorescence and Agglutination. J. Appl. Bact. 55:253-261.

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.

Somasegaran, P., 1985. Inoculant Production with Diluted Liquid Cultures of *Rhizobium* spp. and Autoclaved Peat: Evaluation of Diluents, *Rhizobium* spp., Peats, Sterility Requirements, Storage, and Plant Effectiveness. Appl. Env. Micro. 50(2):398-405.

Somasegaran, P. and Hoben, H.J. 1985. Methods in Legume-Rhizobium Technology. NifTAL 367 pp.

Somasegaran, P. and R.B. Martin, 1986. Symbiotic Characters & *Rhizobium* Requirement of a *Leucaena leucocephala* x *L. diversifolia* Hybrid and Its Parental Genotypes. Appl. Envir. Micro. 52(6): 1422-1424.

Somasegaran, P., H. J. Hoben, and Velittin Gurgun, 1988. Effects of Inoculation Rate, Rhizobial Strain Competition, and Nitrogen Fixation in Chickpea. Agronomy J. 80(1): 68-73.

Somasegaran, P, H.J. Hoben, and J.C. Burton, 1988. A Medium-Scale Fermentor for Mass Culture of Rhizobia. Illustrated Concepts In Agricultural Biotechnology, #3, 8/1988.

Somasegaran, P., R.C. Abaidoo, and F. Kumaga, 1989. Host-*Bradyrhizobium* relationships and nitrogen-fixation in the Bambarra Groundnut [*Voandzeia subterranea* (L.) Thouars nom. cons.]. Tropical Agric. (In Press).

Somasegaran, P., R.-D. Hirtz, and W.J. Broughton, 1989. Rhizobia in Tropical Legumes: Cultural Characteristics of *Bradyrhizobium* and *Rhizobium* sp. Soil Biol. Biochem. (In Press).

van Kessel, C. and P. Nakao, 1986. The Use of Nitrogen-15-Depleted Ammonium Sulfate for Estimating Nitrogen Fixation by Leguminous Trees. Agron. J. 78(3): 549-551.

van Kessel, C., Singleton, P.W., and Hoben, H.J., 1985. Enhanced N-Transfer from a Soybean to Maize by Vesicular Arbuscular Mycorrhizal (VAM) Fungi. Plant Physiol. 79: 562-563

van Kessel, C. and J.P. Roskoski. 1985. Annual, Seasonal, and Diel Variations in Nitrogen Fixing Activity by *Inga jinicuil*, a Tropical leguminous tree. OIKOS 44: 306-312.

van Kessel, C. and J. P. Roskoski. 1988. Row spacing effects on N_2-fixation, N-yield and soil N uptake of intercropped cowpea and maize. Plant and Soil 111: 17-23.

van Kessel, J. P. Roskoski and K. Keane, 1988. Ureide Production by N_2-Fixing and non-N_2-Fixing

Leguminous Trees, 1988. Soil Biol. Biochem 20(6): 891-897.

Vincent, J.M. (Ed.) et al, 1977. Proceedings of a Workshop on 'Exploiting the Legume/*Rhizobium* Symbiosis in Tropical Agriculture', 470 pp.

Welsch, D.E., 1982. Economic Analysis of Biological Nitrogen Fixation. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

Welsch, D.E., and M.R. Kilkenny, 1982. The Contribution of Forages to Farm Earnings in Southeastern Minnesota. Agron. Abst. p. 130.

Whitney, A.S., 1982. Legumes for Hawaii Pastures. Proceedings: 17th Mealani Beef Cattle Field Day, Mealani Expt. Stn., Kamuela, Hawaii.

Whitney, A.S., 1982. The Role of Legumes in Mixed Pastures. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical.

Whitney, A.S., 1983. Contributions to the N Economy of Both Wheat and Maize Due to Legume Intercropping. Proceedings of Workshop on 'Nitrogen Dynamics for Tropical Cropping Systems'. Rodale, Penn.

Whitney, A.S., Koch, B.L., and T.J. Wacek, 1976. Nitrogen Fixation by Tropical Legumes. Proceedings, 1st Review Meeting of the INPUTS Project. Honolulu, Hawaii.

Woolfenden, R.B. and B.B.Bohlool, 1983. Population Dynamics of *Rhizobium japonicum* and *Rhizobium leguminosarum* in Host and Non-host Rhizospheres. 9th North American Rhizobium Conference, Ithaca, New York.

Woomer, P.L., 1979. Root Tuberization and Nitrogen Fixation by *Pachyrhizus erosus*. M.S. Thesis, Univ. of Hawaii, 96 pp.

Woomer, P., Guevarra, A., and K. Stockinger, 1978. Winged Bean Investigations at NifTAL: Observational Garden *Rhizobium* Strain Testing and Response Across a Liming Gradient. In. *The Winged Bean: Papers Presented in the 1st International Symposium on Developing the Potentials of the Winged Bean.* Philippine Council for Agriculture and Resources Research.

Woomer, P., P.W. Singleton, and B.B. Bohlool, 1988. Ecological indicators of native rhizobia in tropical soils. Appl. Env. Micro 54(5): 1112-1116.

Woomer, P.L., P.W. Singleton, and B.B. Bohlool, 1988. Reliability of the Most-Probable-Number Technique for Enumerating Rhizobia in Tropical Soils. Appl. Env. Micro. 54(6): 1494-1497.

Woomer, P. L. and B.B. Bohlool, 1989 Rhizobial Ecology in Tropical Pasture Systems. In. Marten et al (Eds.), *Persistence of Forage Legumes*. ASA, Madison, WS, p. 233-248.

Woomer, P., J. Bennett, R. Yost, 1989. Overcoming the Inflexibility of Most Probable Number Procedures. Agronomy J. (In Press)

Woomer, P., W. Asano, and D. Pradhan. Environmental Factors Related To Rhizobial Abundance in Kikuyugrass (*Pennisetum clandestinum*) Pastures. Tropical Agric. (In Press)

Zaroug, M.G. and D.N. Munns, 1979. Nodulation, Nitrogen Fixation, Leaf Area, and Sugars Content in *Lablab purpureus* as Affected by Sulfur Nutrition. Plant and Soil 53:319-328.

Zaroug, M.G. and D.N. Munns, 1979. Nodulation and Growth of *Lablab purpureus* in Relation to *Rhizobium* Strain, Liming, and Phosphorus. Plant and Soil 53:329-339.

Zaroug, M.G., and D.N. Munns, 1980. Effects of Phosphorus and Sulfur Nutrition on Soluble Sugars and Growth in *Clitoria ternatea* L. Plant and Soil 55:243-250.

Zaroug, M.G. and D.N. Munns, 1980. Influence of Phosphorus and Sulfur Nutrition on Composition of *Clitoria ternatea* L. Plant and Soil 55:251-259.

Zaroug, M.G. and D.N. Munns, 1980. Screening Strains of *Rhizobium* for the Tropical Legumes *Clitoria ternatea* and *Vigna trilobata* in Soils of Different pH. Trop. Grasslands. 14:(1):28-33.

Project Personnel

Scientific Staff

B. Ben Bohlool, Ph.D., Director, NifTAL Project

Nantakorn Boonkerd, Ph.D., BNFRC Director (Thailand)

Robert Davis, Ph.D., Consultant

Heinz Hoben, M.S., Foreign Training Coordinator

Harold H. Keyser, Ph.D. (Oct. -), Plant/Soil Microbiologist

Francois Robert, Ph.D. (May-June), Assistant Professor

Joann Roskoski, Ph.D. (Jan-May), Associate Project Director; Latin America Coordinator

Paul Singleton, Ph.D., Head of Research; Assistant Agronomist; Network Coordinator

Padma Somasegaran, Ph.D., Microbiologist/Training Course Director

Wendy Asano, M.S., Research Associate

Candy Barr, B.S. (Jan-June), Laboratory Assistant

Ed Coughlin, B.A. (Dec -), Research Associate

Brion Duffy (June-August), Temp Summer Research Associate

Geoffrey Haines, Field Assistant

Janet Hudson, B.S. (Jan-March), Research Associate

Kevin Keane, B.S., Research Associate

Kathy MacGlashan, B.S., Research Associate

R. Bruce Martin, B.S., Research Associate

Patty Nakao, B.S., Research Associate

Dawn Olsen, B.S. (Jan-Aug), Research Associate

Anong Rattanoubol, B.S., (Jan-March), Reseaarch Associate

Joseph Rourke, B.S., Research Associate

Lalana Sansopha, B.S. (Jan-July), Research Associate

Patricia Scott (June -), Laboratory Assistant

Yaowanuch Singhathep, B.S., Research Assistant (Thailand)

Jintana Thongu, Laboratory Assistant (Thailand)

Rungtip Tinnachartvanit, M.S., Research Assistant (Thailand)

Thomas Walker,Field Assistant

Paul Woomer, M.S., Research Associate

Support Staff

Princess Ferguson, B.P.A., Head, Communication Section

Benjaluck Lertbhuddhikul, B.B.A. (Jan-May) Information Officer, (Thailand)

Nartkamol Pawai, B. Econ, Secretray (Thailand)

Karean Zukeran, A.A., Project Manager

Lionel Amoral Jr., (Dec. -), Groundskeeper

John Blair (Dec -), Temporary Professional Carpenter

Bhiromat Bhupanthong, B.B.A. (Jun -), Typist (Thailand)

Richard Gabrielson (Jan-Sept), Graphic Artist

Christine Goray (Sept-Dec), Graphic Artist/Photographer

M. Anna Gillies (Oct -), Graphic Artist

Susan Hiraoka, Clerk Typist

Pam Hoopii, Utility Person

Sally Hoopii (Feb-April), Typist/Computer Operator

Chonticha Jansuda, Office Maid (Thailand)

Patricia Joaquin, Clerk Typist

Mack Jones, Maintenance

Arthur Lee (Jan-Nov), Maintenance (Part-time)

Harold Lee, Groundskeeper

Vickie Machoi (Sept-Dec), Temp Clerk Typist

John McCollum (Dec -), Temp Carpenter Assistant

Ade Nikaido, Account Clerk

Aroon Rattanamaneekul, Janitor (Thailand)

Susan Tomoso (March-Dec), Office/Library Clerk & Asst Workshop Organizer/Secretary

Michael Unverzagt (Jan-Jul), Maintenance Person

Graduate Students

M.Sc. Degree Candidates

Tanya Thongnunui (Thailand)

Ph.D. Degree Candidates

Thomas George

Janice Thies

Maria Luz Caces

Dan Turk (August -)

International Travel

1988

Bohlool, Ben
Germany
March 08-21, 1988

Bohlool, Ben
Acapulco, Mexico
May 15-20, 1988

Bohlool, Ben
India, Thailand, Philippines
July 20-28, 1988

Bohlool, Ben
Pakistan, Thailand
September 01-11, 1988

Boonkerd, Nantakorn
Acapulco, Connecticut, Germany
February 19 - March 05 - March 12-21, 1988

Boonkerd, Nantakorn
Maui, HI, Connecticut, Washington
August 01-18, 1988

Davis, Robert
Sri Lanka
April 14-July 01, 1988

Singleton, Paul
India, Bangkok
July 19-27, 1988

Singleton, Paul
Uganda
November 26-December 20, 1988

Somasegaran, Padma
Hong Kong
July 30-August 06, 1988

1989

Bohlool, Ben
Bangkok, Viet Nam
January 08-20, 1989

Boonkerd, Nantakorn
Los Banos
July 09-12, 1989

Hoben, Heinz
Bangkok
October 23-November 29, 1989

Keyser, Harold
Port-au-Prince
July 12-14, 1989

Singleton, Paul
Bangkok, Manila
March 02-April 05, 1989

Singleton, Paul
Indonesia (consultancy)
May 14-June 20, 1989

Singleton, Paul
Manila
July 08-21, 1989

Singleton, Paul
Kampala
October 14-November 13, 1989

Somasegarsan, Padma
Bangkok, Malaysia
October 23-December 06, 1989