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ANNUAL REPORT 1991

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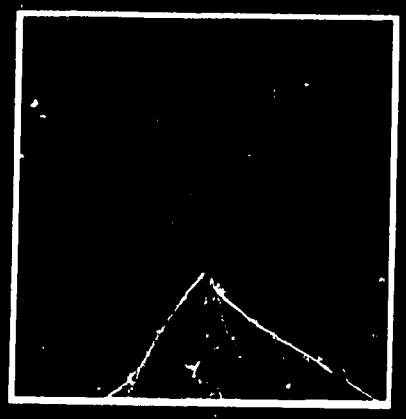

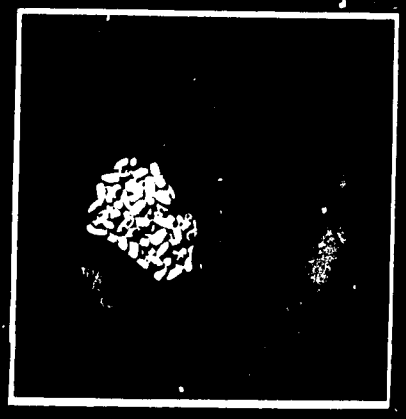

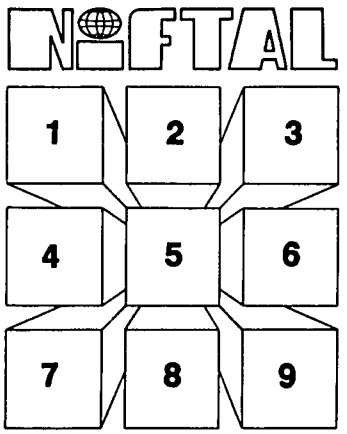

1. Senior legume scientists learn advanced techniques in NifTAL's intern program.

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

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

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

5. Inoculated bean seeds on the right have the edge - 10,000 rhizobia per seed.

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

7. A 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 1991

NifTAL Project and MIRCEN

A Report for 1/1/90 - 9/30/91

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

Dr. B. Ben Bohlool and Dr. Paul Singleton
Principal Investigators

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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 Investigators:	Dr. B. Ben Bohlool/Dr. Paul Singleton
Address:	NifTAL Project and MIRCEN 1000 Holomua Road 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:	01-01-90 to 9-30-91
Total Budget:	$4,800,000
Obligated Funds to Date:	$1,930,000
Project Office:	R&D/AGR/RNR
Project Monitor:	Dr. Charles Sloger R&D/AGR/RNR, SA-18 U.S. Agency for International Development Washington, D.C. 20523

Acknowledgements

The University of Hawaii acknowledges the continuing support by the United States Agency for International Development for the NifTAL Project. This USAID core support for NifTAL has permitted the establishment of an integrated, multi-disciplinary program augmented by additional awards from public, private, and international sources. NifTAL acknowledges the funding that these grantors have provided directly to the Project and to NifTAL's visiting scientists and trainees. These grantors include the National Science Foundation, MIRCEN, UNESCO, United States Department of Agriculture, the Science Advisor's Office-AID, the McIntire-Stennis Foundation, OECD, African American Insitute, San Diego State University, IRRI, IITA, UNFAO, and the Thai Department of Agriculture.

We recognize the support of the Mayor and County Council of Maui for provision of the 22.2 acre NifTAL research campus. NifTAL is also indebted to the community of Maui which has fostered the Project by providing a dedicated staff, field sites, and encouragement and assistance with Project activities. The State of Hawaii has continued to award monies to the University for renovations at NifTAL, while the University of Hawaii has provided NifTAL with administrative support, staff positions, and equipment items.

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B. Ben Bohlool
In Memoriam

The BNF community throughout the world was shocked and saddened by the death of Dr. B. Ben Bohlool, NifTAL Director, on May 16, 1991. Ben died as a result of an automobile accident that occurred on his way to work. Funeral services were held on the grounds of the NifTAL Project site in Hamakuapoko, Maui, Hawaii on May 21.

Ben was born in Tehran, Iran, in 1940. In 1971, he graduated with a Ph.D. in microbial ecology from the University of Minnesota at Minneapolis under Dr. E. L. Schmidt. His doctoral work focused on the use of immunofluorescence in the study of the ecology of *Rhizobium japonicum* in soils. Further post-doctoral research with Dr. Schmidt resulted in their formulation of the lectin hypotheses. This pioneering work suggested that lectins were a possible basis for specificity in the legume-rhizobia symbiosis.

Ben also conducted post-doctoral research at the University of Wisconsin under Dr. T. D. Brock studying the ecology of microorganisms inhabiting thermal and acidic conditions. In 1974, he was appointed Research Scientist at the Cawthron Institute in New Zealand to investigate the microbial aspects of water pollution and eutrophication. While in New Zealand he patented a process for making protein-binding microspheres.

In 1976, Ben joined the faculty of the University of Hawaii as an Assistant Professor of Microbiology and advisor to the NifTAL Project. He was promoted to Associate Professor of Microbiology in 1980 and to Professor in 1984. His research at the University of Hawaii encompassed several major areas including the ecology of rhizobia in tropical soils, assessing the potential of fast-growing soybean rhizobia form China, and studying the host regulation of the legume-rhizobia symbiosis. During his tenure at the University of Hawaii, Ben was the recipient of the Alexander von Humboldt Fellowship, and spent six months with the molecular genetics group at Max-Planck Institute in Cologne, Germany.

In 1985 Ben was appointed Director and Principal Investigator of the NifTAL Project. As NifTAL's Director, Ben fostered his vision of the Project as both a dynamic research institute as well as a responsive resource for promoting BNF in agricultural development. He led the Project in developing international outreach and training activities, and was nominated for the World Food Prize for these efforts. Ben also remained active in research, and supported an international network of researchers in rhizobial ecology to study environmental effects on the occurrence, persistence, and performance of rhizobia in tropical soils.

Ben is remembered by his friends and colleagues as an excellent and creative scientist. He was blessed with an insatiable curiosity, social gregariousness, and the ability to savor all aspects of life. Besides his own scientific contributions, Ben's legacy includes the continuing work of his graduate students, and that of his NifTAL "family." All of us who were fortunate to know and work with Ben have been indelibly influenced by both his intellect and his passion for life.

Ben is survived by his wife, Carol, and their fourteen-year-old daughter, Kiaora, who will continue to reside on Maui. At the memorial service, Kiaora Bohlool shared the following poem which she found in her father's briefcase on the day of his death.

Though nothing can bring back
The hour of splendor in the grass
And the glory of the flower,
We shall grieve not;
Rather find strength in things we leave behind.

Overview

Abbreviations and Acronyms

AID Agency for International Development

ACCLAIM Accounting for Laboratories by Automation and Information Management

BNF Biological Nitrogen Fixation

BNFRC BNF Resource Center (Thailand)

BNF/LMOPP Biological Nitrogen Fixation/Legume Management Outreach Pilot Project

BOSTID Board on Science and Technology for International Development (National Academy of Sciences)

CIAT Centro Internacional de Agricultural Tropical

CRSP Cooperative Research Support Program

ELISA Enzyme Linked Immunosorbent Assay

FA Flourescent Antibody Technique

FAIME Financial Analyses for Inoculant Manufacturing Enterprises

FUE Fertilizer Use Efficiency

IAB Institute of Agriculture Burma

IAEA International Atomic Energy Agency

IBSRAM International Board for Soil Research and Management

ICARDA International Center for Agricultural Research in the Dry Areas

IFDC International Fertilizer Development Center

IITA International Institute for Tropical Agriculture

INDO/US STI India/United States Science and Technology Initiative, USAID S&T

INLIT International Network of Legume Inoculation Trials

IRRI International Rice Research Institute

IS Insertion Sequences

LDCs Lesser Developed Countries

MARDI Malaysian Agriculture Research and Development Institute

MFAD Manpower for Agriculture Development Project, Kampala Uganda

MIRCEN Microbiological Resource Center (UNESCO designation)

MPN Most Probable Number

NFE Nitrogen Fertilizer Efficiency

NFTA Nitrogen Fixing Tree Association

NGO Non-Government Organizations

NifTAL Nitrogen Fixation by Tropical Agricultural Legumes

NSF National Science Foundation

PCARRD Philippine Council for Agriculture Resources Research and Development, Los Banos, Philippines

PSTC Program in Science and Technology Cooperative

PTRTC Philippine Tobacco Research and Training Center

PVO Private Voluntary Organizations

RFLP Restriction Fragment Length Ploymorphism

S&T/AGR Bureau for Science and Technology/Office of Agriculture (AID)

TROPSOILS Tropical Soils Research Network

UH University of Hawaii

UNEP United Nations Environment Program

UNESCO United Nations Education and Science Organization

UPLB University of the Philippines, Los Banos

USAID U.S. Agency for International Development

VAM Vesicular Arbuscular Mycorrhizal Fungi

WREN Worldwide Rhizobial Ecology Network

ZAMARE Zambia Agricultural Research and Extension Project

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Legume Biological Nitrogen Fixing Symbiosis Resources for Sustainable Development

The Challenge

- Rapid population growth in the developing tropics has created an urgent demand for increased food and fuelwood production.
- Nitrogen is the most common limiting soil nutrient needed to increase agricultural production in the tropics.
- Chemical sources of nitrogen are produced by non-renewable, petroleum based energy sources. They are generally expensive or unavailable to farmers in developing countries. These fertilizers are also often associated with pollution of water resources due to runoff.
- Many farmers and extension specialists in developing countries are unaware of the benefits of biological sources of nitrogen.
- Agricultural expansion and resource exploitation in the tropics has often resulted in destruction of natural ecosystems, soil degradation, and erosion.

The Potential

- Nitrogen inputs through BNF support sustainable agricultural systems.
- Legume species provide high protein grains for human consumption, forage for animal production, and fuelwood. Legume species can also be used for restoration and stabilization of degraded lands.
- Biological nitrogen fixing symbioses between legumes and rhizobia allow the legumes to flourish in nitrogen deficient soils without applying nitrogen fertilizers.
- Intercropping and relay cropping with legumes benefit the non-legumes in the cropping system.
- Nitrogen fixed by legumes is cost-free if appropriate rhizobia are already present in the soil.
- If rhizobia are not already present in the soil, introducing rhizobia by seed inoculation is simple, inexpensive, and energy efficient.
- BNF is cost effective. In nitrogen deficient soils, it would take approximately US$87 worth of urea to produce a soybean yield comparable to that possible using only $3 worth of inoculant.
- BNF can contribute to increased yields and farm incomes, improved diets, and community development.

Rhizobia infect their legume root host's roots and cause the formation of root nodules. The extensive nodulation on this root system indicates the plant is benefitting from symbiotically fixed nitrogen.

The Tasks

- **Needs Assessment:** Not all legumes capable of fixing nitrogen do so in every situation. An important task is to conduct an assessment of the potential for BNF in a given environment. Another is to refine the tools for determining when, where, and with which crops BNF can be enhanced by seed inoculation.

- **Technology Transfer:** Seed inoculation technology has been used extensively in developed countries for many years. Transferring this technology to areas with limited resources and conditions where BNF can make the most impact will require a sustained effort. Linkages between institutes generating the technology and those charged with its dissemination must be established and strengthened.

- **Education:** Education is the foundation for technology transfer. Building awareness of the potential for BNF in cropping systems among government planners is a critical first step. Further training of local scientists, extension specialists, and private voluntary organization personnel is necessary to develop conduits for knowledge and technology transfer.

- **Production Resources:** The development of the facilities and personnel to produce inoculants and the infrastructure to distribute the product must be linked to the education process. The eventual goal is the establishment of inoculant industries which respond to the market demands of the farmers.

- **Adapting the Technology:** Applied research to make BNF technology simpler, more cost effective, and efficient will facilitate technology transfer. Research is also needed to ensure quality of inoculants under the specific conditions where they are produced and used.

- **Identifying Management Practices to Maximize BNF:** Continuing research is needed to identify management practices which maximize BNF in cropping systems. These research efforts address environmental constraints to BNF, as well as the integration of legume components into traditional cropping systems, and establishing effective agroforestry strategies.

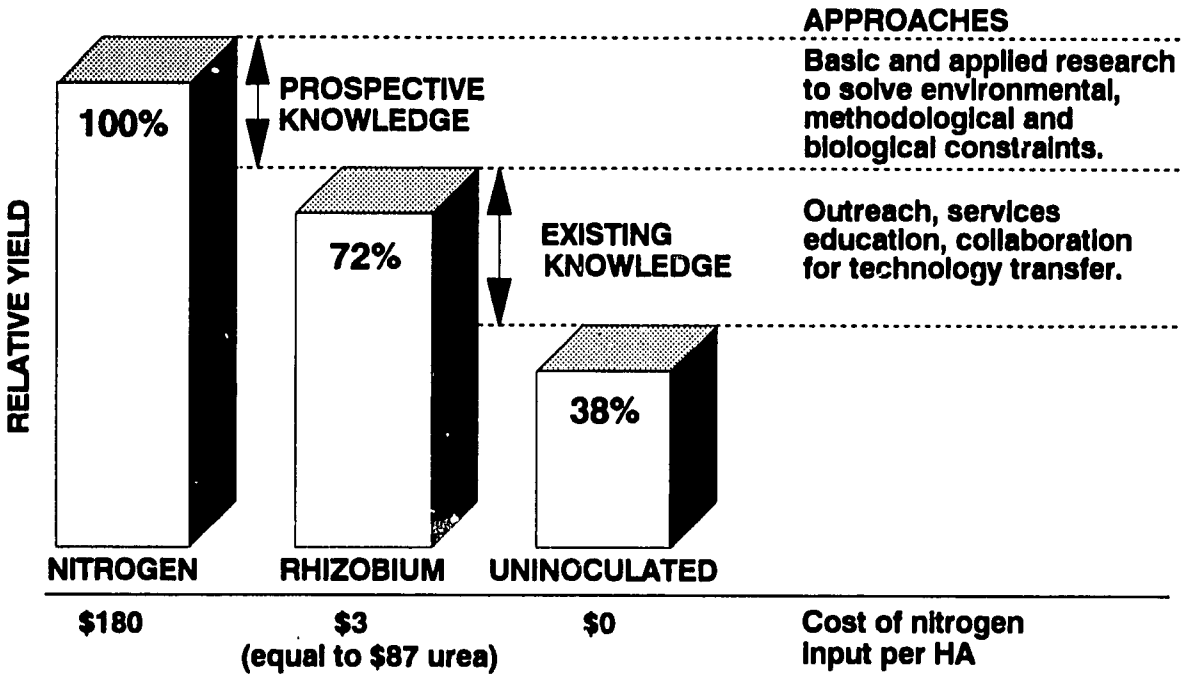
- **Improving the Symbiosis:** Enhancing the nitrogen fixing symbiosis requires understanding the dynamics of the host-microbe interaction, and the biological constraints to this partnership. Research can also identify superior host-strain combinations for inoculant formulations. Finally, knowledge about the genetic determinants of the symbiosis is necessary for eventual manipulation of this complex relationship.

This figure depicting relative yields of legumes is derived from five field inoculation trials conducted in Maui, Hawaii. The "100%" bar represents the maximal genetic yield potential of the crop under ideal conditions when receiving approximately 1000 kg N per hectare. This level of N fertilization necessary to obtain the highest yield potential is both economically and functionally impractical. The second "72%" bar represents the proportion of the maximal yield which can be obtained when using rhizobial inoculants. The cost of the inoculant is approximately $3, while the yield is equivalent to that obtained when using approximately $87 worth of chemical nitrogen fertilizer. The third "38%" bar shows the relative yield of crops receiving neither fertilizer nitrogen nor inoculant. This is the relative yield obtained when the crop was dependent solely on native soil nitrogen resources.

Increasing yields through inoculation requires the application of an existing seed inoculation technology. NifTAL's outreach efforts include education, information and materials dissemination, product and facilities development, and collaboration for transferring this technology.

Bridging the gap between the yield obtained by inoculation and the maximal yield potential requires both basic and applied research efforts. NifTAL continually strives to maximize the benefits of BNF through genetic manipulation of the partners, identifying more efficient host-microbe combinations, defining appropriate management techniques, and solving environmental and biological constraints to the symbiosis.

The Potential for BNF



Background

In 1975 at the peak of the oil crisis, national economies worldwide contended with the high costs of fossil fuels. The nitrogen fixing symbiosis between legumes and rhizobia was recognized as an attractive, low cost alternative to petroleum based nitrogen fertilizers for increased crop production in the tropics. The United States Agency for International Development (USAID) drew on expertise in the University of Hawaii's College of Tropical Agriculture and Human Resources to establish 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. NifTAL's ultimate purpose was to help farmers maximize BNF inputs to their cropping systems, and thereby increase the production and quality of high protein foods while reducing their dependence on expensive nitrogen fertilizers.

The initial USAID contract (AID/ta-C-1207) ran from June 1, 1975, through June 15, 1982. During this period NifTAL built its resource capability to meet the needs of national legume programs in developing countries for research support, information and technical services, and training in BNF. At NifTAL headquarters at Hamakuapoko, Maui, Hawaii, a rhizobial germplasm resource was collected and tested to identify effective symbionts for 50 target legumes. In 1979, NifTAL initiated a campaign to gather definitive field data on legume response to inoculation with these rhizobia throughout the tropics. A network of more than 200 collaborators participated in NifTAL's International Network of Legume Inoculation Trials (INLIT) to conduct standardized experiments in more than 50 tropical countries. This effort verified the benefits of legume inoculation in many parts of the tropics, and promoted global awareness of BNF.

From June 16, 1982, through December 31, 1986, (USAID Contract DAN-O613-C-OO-2064-00), NifTAL continued to develop appropriate BNF technologies for tropical agriculture. Technology transfer and training activities increased, particularly those related to production and use of inoculants. In 1983, the BNF Resource Center (BNFRC) for South and Southeast Asia was established to extend NifTAL's efforts through a regional center. During this contract period, NifTAL also initiated WREN (Worldwide Rhizobial Ecology Network), a select group of researchers conducting standardized field trials linking the response to inoculation to measurable environmental parameters.

USAID continued to support NifTAL through a Cooperative Agreement (DAN-4177-A-00-6035-00, September 30, 1986 to September 29, 1991) known as Improved BNF Through Biotechnology. This award acknowledged 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 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; 5) Commercial Inoculant Technical Assistance.

During this recent contract period major progress was made in the development of models to predict where and to what extent legumes will respond to inoculation. NifTAL also strengthened its capacity to transfer BNF technology to extension personnel, and thereby forged another conduit through which NifTAL expertise can serve developing country farmers. Another avenue of technology transfer is the ongoing development of services to private and government inoculant production enterprises.

This reporting period extends from January 1, 1990 to the completion of the recent cooperative agreement with USAID on September 30, 1991. On October 1, 1991, NifTAL entered a new three year cooperative agreement with USAID. As we face massive population growth in the tropics and global environmental degradation, the present need for BNF technology in developing countries is critical. NifTAL's initial goal to promote BNF for the welfare of farmers in the developing tropics continues to be relevant today as BNF is recognized as a cornerstone of low input sustainable agricultural systems. In addition, BNF is being exploited to benefit agroforestry systems, soil conservation, and ecosystem restoration programs. Today, NifTAL continues as an important resource to meet the challenges of sustainable agricultural development and the preservation of environmental quality in the tropics.

Summary of Accomplishments

This reporting period, January 1, 1990 to September 30, 1991, was a productive time for the NifTAL Project as we continued to face the challenges of exploiting BNF for international development. During this period we strengthened our collaborative linkages with international agricultural institutions in Asia and Africa to address problems of agricultural sustainability in the tropics. Outreach and educational activities were emphasized to promote BNF technology transfer. Research was also a strong component of our project. Research efforts addressed environmental, biological, and methodological constraints to maximizing BNF. In addition, our scientific endeavors fostered the development of useful technologies and products to facilitate BNF technology transfer, especially via commercial enterprise.

Highlights of our accomplishments include:

Networking and Collaboration

NifTAL continued its participation in the Microbiological Resource Center (MIRCEN) network of institutions charged with the preservation of rhizobial strains, the Worldwide Rhizobial Ecology Network, and the network of private voluntary organizations involved in the BNF Legume Management Program. We also strengthened our collaboration with the International Agricultural Research Centers by placing research staff at both the International Rice Research Institute (IRRI) in the Philippines and at the International Institute for Tropical Agriculture (IITA) in Nigeria. We collaborated with various other agricultural institutes in the Philippines and Malaysia to conduct on-farm trials and germplasm screening, respectively.

Technical Assistance and Technology Transfer

NifTAL's BNF Resource Center again provided valuable outreach services to the Asian region. BNFRC Director Dr. Nantakorn Boonkerd provided rhizobial strains, inoculants, training, and consultancies to institutes in the region. His technical assistance was especially valuable to inoculant production in Thailand, Indonesia, and Bhutan. NifTAL staff also established an inoculant production facility in Uganda, and provided materials and technical assistance to increase the inoculant production capacity of Zambia's facilities. Back in the U.S., we learned that efficient, low cost production methodologies developed by NifTAL were also adopted by a domestic inoculant manufacturer.

Students in NifTAL BNFRC extension course held in Khon Kaen, Thailand, March 1990

Material and Informational Services

A new aspect of NifTAL's services was a program to test indigenous materials for their suitability as inoculant carriers. In response to requests from around the globe, we also distributed more than 1000 cultures, 900 packets of inoculants, and 150 mls of high quality antisera. Our information services reached individuals and institutions with interests in BNF in more than 100 countries. We provided news bulletins, a continuing bibliography, reprints, manuals, software, and technical information. A major accomplishment of our information service was the development of a modular resource manual with supplementary training materials for training extension specialists. This manual has also been translated into Indonesian.

Education

Education efforts are a key component of technology transfer. NifTAL developed a practical training course for extension specialists who will in turn transmit their knowledge to other extension agents and farmers. We conducted training courses for Indonesian and Ugandan extension specialists using our new resource manual as a guide. We also conducted our first training course for North African scientists in Morocco, and our basic training in rhizobiology for South Asian scientists in Thailand. NifTAL also hosted the Indo-US Science and Technology Workshop on Maui, and long term visiting scientists from Slovenia, Germany, Turkey, and Madagascar. During this period, NifTAL graduated three Ph.D. students, and is now supporting Ph.D. candidates from Guyana, Nigeria, Trinidad, and the U.S., and a Masters student from Argentina.

Research Addressing Environmental Constraints to BNF

An ambitious collaborative research program addressing the conservation and use of soil and fixed nitrogen through legumes in lowland rice-based cropping systems was initiated in the Philippines. This important effort combines the expertise of NifTAL, IRRI, and the International Fertilizer Development Council (IFDC). NifTAL also cooperates with Philippine government agencies to conduct on-farm trials to optimize rice-based cropping sequences. We also worked with the Malaysian Agricultural Research and Development Institute (MARDI) to develop acid tolerant leucaena germplasm and rhizobia. Other research concentrated on the phosphorus economies of fixing and non-fixing legumes in phosphorus deficient soils common to the tropics.

Children, like this boy from Togo, are the most precious beneficiaries of improved dietary protein from BNF.

Research Addressing Host-Rhizobial Interactions

During this reporting period we completed a major program defining the rhizobial specificity of a range of leguminous trees used in agroforestry programs. This information has been valuable for the formulation of inoculants for those species. Other research addressed the regulation of nodulation by the host from the perspectives of both host physiology and microbial competence. Collaborative research between NifTAL and IITA has also been initiated to address the rhizobial needs for legume germplasm being developed for Africa.

Methodologies for Understanding BNF

Methodologies for enumerating rhizobia for specific leguminous trees were defined. Our biotechnology program developed a highly sensitive method for identifying *Rhizobia* sp., and also identified and cloned the symbiotic region of a rhizobial strain with superior symbiotic and competitive properties.

Tools for Development

We have been especially proud of the cost-effective products and techniques we have developed this period to facilitate adoption of BNF technologies. Outputs include computer software. Our program RESPONSE uses ecological models to predict the response to legume inoculation in a given area. This program assists decision makers and regional planners to use limited BNF resources judiciously. Another program, FAIME, provides financial analyses for prospective inoculant manufacturers. ACCLAIM is a data base of rhizobial germplasm collection and recommended seed treatment protocols. Another product is our modular training guide on applied BNF for extension specialists. This guide has also been translated into Indonesian. NifTAL's fermentors, low cost media formulations, enumeration systems, and antisera production methods are all useful to ensure the quality of inoculants and the financial sustainability of inoculant enterprises, while our improved biotechnology methods can reduce expenses of research programs in developing countries.

Outreach and Education

NifTAL's outreach and education activities focus on the delivery of BNF technology to our clients overseas. NifTAL and the BNF Resource Center in Bangkok, Thailand, implement complementary programs for technology transfer. These activities are primarily directed toward research institutes, extension specialists, and commercial enterprises in developing countries. Avenues for delivering this technology include networking, collaborative research, workshops, training, technical assistance, and material and information services.

NifTAL has recognized the need to participate in both formal and informal networks for collaborative research throughout the Project's history. Scientists from diverse areas of the tropics have contributed to the development of tools for more predictable and focused transfer of BNF technologies. NifTAL's International Network of Legume Inoculation Trials (INLIT) of the early 1980's demonstrated the potential for BNF at more than 200 sites across the tropics. INLIT was followed by the Worldwide Rhizobial Ecology Network (WREN) of selected scientists in 18 countries who correlated responses due to legume inoculation with measurable environmental parameters. This research has led to the development of models to predict the yield increases to legume inoculation in any given site.

NifTAL is now networking with International Agricultural Research Centers. NifTAL staff have been placed at the International Rice Research Institute in the Philippines and the International Institute for Tropical Agriculture in Nigeria to address specific regional concerns. Extramural grants have fostered collaboration with other research institutes in Malaysia and the Philippines.

In addition to scientific collaboration at the institutional and professional levels, NifTAL also delivers applied technologies. Participation in the BNF Legume Management Outreach Pilot Program directed NifTAL's expertise into field level PVO/NGO networks in Haiti and Uganda. Technical assistance and training are also transferred to inoculant producers and extension specialists. NifTAL developed fermentors and low cost technologies have catalyzed commercial and government inoculant production at sites in Asia and Africa. Another major output of NifTAL's outreach efforts was the development of an applied BNF training program, manual, and promotional materials for extension specialists. Extension specialists with a thorough understanding of BNF are critical for promoting the inoculants to farmers and developing the market for commercial producers.

The visiting scientist and graduate student programs are other important components of NifTAL's educational agenda. NifTAL hosts visiting scientists who learn new techniques while they conduct cooperative research with NifTAL staff. This exchange strengthens the visitors' understanding of the symbiosis and stimulates BNF programs in their own institutions. NifTAL graduate students have made important advances to our scientific knowledge base while discovering practical solutions to problems in BNF. These graduates have continued to contribute to this field as researchers, program leaders, and educators.

From NifTAL headquarters in Hawaii, material and informational services are extended to more than 100 countries through inoculants, cultures, news bulletins, and bibliographies. These services range from providing highly technical material for scientific research, to evaluating inoculant carriers for commercial producers, to answering questions from farmers and rural community leaders.

NifTAL has also developed a variety of interactive computer programs to facilitate technical and planning processes. For example, an automated rhizobial germplasm catalogue is valuable for the selection of strains for inoculant production. The RESPONSE program predicts legume yield responses for a site, based on environmental parameters such as soil nitrogen status and cropping history. Another example is the FAIME program which provides a financial analyses for prospective inoculant manufacturing enterprises.

NifTAL's outreach and education program is an integrated effort to promote BNF in the areas most in need of this low cost, high return technology. We have the capability to disseminate the knowledge and the tools to developing country researchers, extension specialists, and entrepreneurs. Our goal is to develop the linkages which take the technology into the farmers' fields. There, BNF can make a difference through increased yields and economic returns, improved diets, and sustained productivity.

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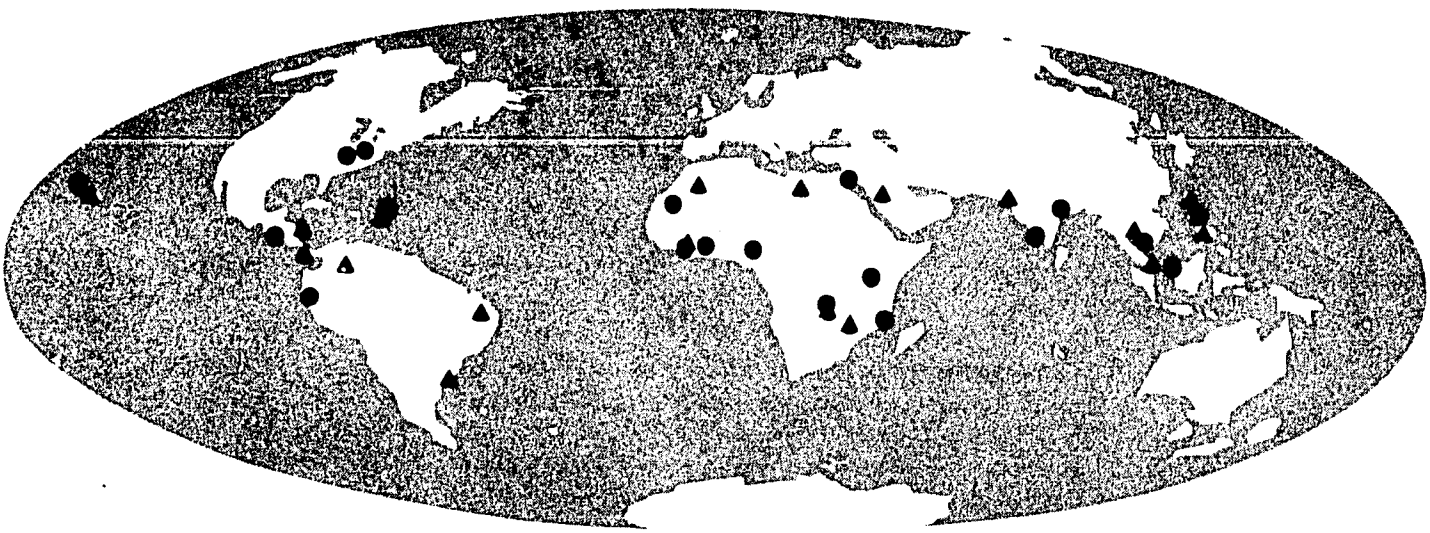
Networking and Collaboration



Reaching out in collaboration and networking (▲) and through technical assistance (●)

Worldwide Rhizobial Ecology Network

Why do some legumes respond to inoculation at certain sites but not at others? NifTAL scientists hypothesized that the reasons could be linked to measurable environmental parameters. In 1986, NifTAL initiated the Worldwide Rhizobial Ecology Network (WREN) to address this question. BNF scientists in 18 countries were invited to participate in this collaborative network. They conducted standardized pot and field legume inoculation trials, and monitored the conditions which affected their results. These standardized WREN trials were also conducted on nine legumes at five ecologically diverse sites on Maui.

As a result of this effort, specific measurable environmental factors were identified as indicators of inoculation response. Mathematical models were formulated to predict where inoculation with rhizobia would result in increased yield based on measurements of the native rhizobial populations and soil mineral N status. These models have been incorporated into first generation decision support systems (see **Tools for Development** section) to aid regional planners considering the establishment of inoculant production facilities.

Both NifTAL and WREN participants benefitted from this scientific collaboration. WREN collaborators contributed their data to strengthen and verify NifTAL's predictive models. In turn, support from the National Science Foundation for WREN assisted many of the collaborators' graduate students to conduct this study.

Furthermore, the concepts which have emerged from WREN have also been a powerful influence on the way the international scientific community views the response to inoculation. WREN has catalyzed a surge of research in developing countries which have used WREN protocols to correlate yield increases due to inoculation with native rhizobial populations and soil N status. By using the scientific premises and protocols of the WREN effort, these researchers have gained the tools to assist inoculant manufacturers in targeting regions for development.

The BNFRC in Thailand

The NifTAL Biological Nitrogen Fixation Resource Center for South and Southeast Asia (BNFRC) was initiated in September, 1983, with the collaboration of the Thailand Department of Agriculture (DOA). The goal was to establish a regional center with a strong foundation for research, training, technical aid, and information dissemination. Because it is oriented to the needs of the region, the BNFRC has been a vital link to institutions in the various countries of South and Southeast Asia engaged in BNF research and development projects.

BNFRC Director Dr. Nantakorn Boonkerd maintains an office, laboratories, and inoculant production facilities in the Thai DOA Rhizobium Building. BNFRC's core of trained personnel supply services such as rhizobial strains and antisera, inoculants, and special analyses. Research projects are conducted in collaboration with regional scientists. The center also provides short-term internships and post graduate programs for developing country scientists to work on various aspects of applied BNF.

Since its establishment, the BNFRC has offered training courses for scientists and extension workers, established linkages with international centers and development organizations, provided internship training, published *A Pocket Manual for Thai Use of Legume Inoculants*, and organized area meetings and conferences.

BNFRC activities during this reporting period included the organization and hosting of training courses and meetings, provision of consultancies and technical assistance to developing country inoculant production efforts, and collaborative research to study legume inoculation responses in tropical cropping systems. Highlights of these activities include:

Technical assistance in rhizobial technology and field design to assess nitrogen fixation in the Democratic People's Republic of Korea, February 1 - March 2, 1990, sponsored by IAEA.

Training course on applied BNF technology for extension specialists from March 26 - April 5, 1990 in Khon Kaen. The course was co-sponsored by DOA, USAID Indonesia, SFCDP, Government of Indonesia, NifTAL, and BNFRC.

Technical assistance in rhizobial technology and inoculant production to Anambra State University at Enugu, Nigeria, April 1-21, 1990, sponsored by IAEA.

Technical assistance on inoculant production in Bhutan, July 11-18, 1990, sponsored by Bhutan, IRRI, and IDRC.

Coordination meeting on screening legumes for high yield and nitrogen fixation from May 26-30, 1991, sponsored by IAEA and FAO.

Training course on rhizobium technology at the BNFRC from June 2-29, 1991, sponsored by NifTAL, IAEA, Thai DOA, and BNFRC.

Research project in collaboration with NifTAL and regional scientists on ecologically based models for prediction of legume inoculation requirements in tropical cropping systems, sponsored by USAID PSTC program.

BNFRC Director Nantakorn Boonkerd supervises trainee learning serological techniques for quality control of inoculants.

The MIRCEN: Network of Genetic Resources

NifTAL is one of a network of institutions worldwide designated as Microbiological Resource Centers (MIRCEN), charged with the preservation of rhizobial strains. NifTAL currently has 1911 rhizobial cultures isolated from a vast array of legume hosts growing in diverse environments throughout the world. UNESCO provides seed funds for conservation of this important ecological resource.

The UNESCO MIRCEN provided support for Professor B. Ben Bohlool (NifTAL Director) to participate as co-convener in the MIRCEN'S Round Table on "Microbiology: International Consensus" at the annual (May/1991) American Society of Microbiology meeting in Dallas. In September, Dr. Padma Somasegaran (MIRCEN Director and rhizobia germplasm curator at NifTAL) was supported by UNESCO to make a presentation entitled "The NifTAL Project MIRCEN and its contribution to international cooperation" at the International Symposium on Biotechnologies and Environment, September, 1991, Montreal, Canada. The MIRCEN at NifTAL also provided partial financial support for candidates from China and Vietnam to attend a training course on "Modern Methods for the Study of Rhizobia," held at the BNFRC, Thailand, from June 3-28, 1991.

The Biological Nitrogen Fixation and Legume Management Outreach Pilot Project: A Consortium to Promote BNF

In 1988, NifTAL participated in a workshop with USAID's Office of Agriculture, Bureau for Science and Technology, and the Office of Private and Voluntary Cooperation, Bureau for Food for Peace and Voluntary Assistance. The participants of that workshop recognized the potential for exploiting the training and extension capabilities of private voluntary organizations (PVO) and the Peace Corps (PC) to deliver BNF technologies to farmers in developing countries.

Following the workshop, NifTAL hosted a two week training course in applied BNF technologies for PVO and PC extension specialists. The consensus of the participants was that BNF was a useful technology which could be integrated into their existing programs in agriculture, forestry, and agroforestry. The participants proposed that a consortium be formed between several PVO, PC, and NifTAL. Through their continued efforts after the course, this consortium was funded by AID in 1989 as the Biological Nitrogen Fixation and Legume Management Outreach Pilot Project (BNF/LM OPP).

The potential for BNF outreach through the BNF/LM Project is tremendous because the infrastructure for agricultural extension and field level networking by PVO and NGO groups is already in place. By exploiting the community linkages of these groups, BNF technology can be efficiently disseminated to farmers.

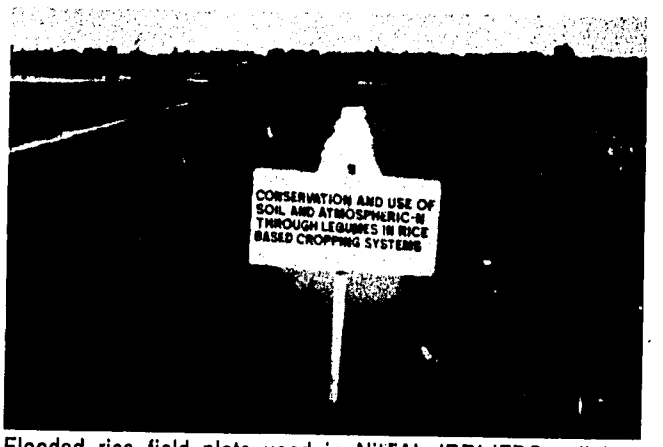
The project has been focusing its efforts on Uganda, Haiti, Nepal, and Senegal. NifTAL staff have been instrumental in seven BNF awareness building workshops for more than 160 representatives of approximately 120 NGO, PVO, government organizations and agribusinesses. The BNF/LM OPP is currently investigating strategies for distributing inoculants in these countries, and demonstration plots have been established in Haiti, Nepal, and Uganda.

IRRI, IFDC, and NifTAL Collaborate to Develop Strategies to Manage Nitrogen in Lowland Rice-Based Cropping Systems

Eighty-seven percent of the 145 M ha of world rice area is in lowlands and much of this is in the tropics. Lowland rice-based cropping systems are characterized by a dry season with aerobic soil conditions, followed by a transition period, and a subsequent wet season when the soil is usually flooded. Nitrogen is the nutrient required in largest quantities in lowland rice-based systems; however, fertilizer N losses are large, and N recovery by flooded rice crops rarely exceed 30%. Furthermore, there are trends of declining rice yields under continuous fertilizer N use. Conserving native soil N from losses and integrating legume BNF in these cropping systems may be viable alternatives to continual dependence on fertilizer N.

NifTAL, the International Rice Research Institute (IRRI) in the Philippines, and the International Fertilizer Development Center (IFDC) are working together on a whole system research approach to develop technologies to conserve and efficiently use nitrogen in the lowland rice ecosystem. This collaborative project "Conservation and Use of Soil and Atmospheric Nitrogen through Legumes in Lowland Rice-based Cropping Systems" was initiated in December, 1989. NifTAL Agronomist Thomas George has been stationed at IRRI where he works with participating scientists Dr. J.K. Ladha and Dr. D.P. Garrity of IRRI and Dr. R.J. Buresh of IFDC.

A conceptualization of the mineral N dynamics in lowland rice are presented in **Figure 1**. The non-flooded dry season favors aerobic nitrogen mineralization that can result in soil NO_3 accumulation. Subsequent flooding results in considerable losses of NO_3 through leaching and denitrification, possibly leading to a net reduction in soil N. The researchers are jointly investigating the N dynamics of the flooded-nonflooded soil sequence, and the impacts of legume BNF on the N economy of these systems. Their initial field trials have focused the potential contributions of legumes and weedy fallows during the non-flooded season to the soil N pool. Because legumes can capture soil N by assimilation as well as atmospheric N through fixation, the use of legumes can increase the efficiency of the cropping systems. Nitrogen in legumes can then be used for direct economic benefit by harvesting as produce, or recycled back to the soil for rice production through the incorporation of residues. Preliminary results are detailed in the research section of this annual report.



Flooded rice field plots used in NifTAL IRRI-IFDC collaborative research

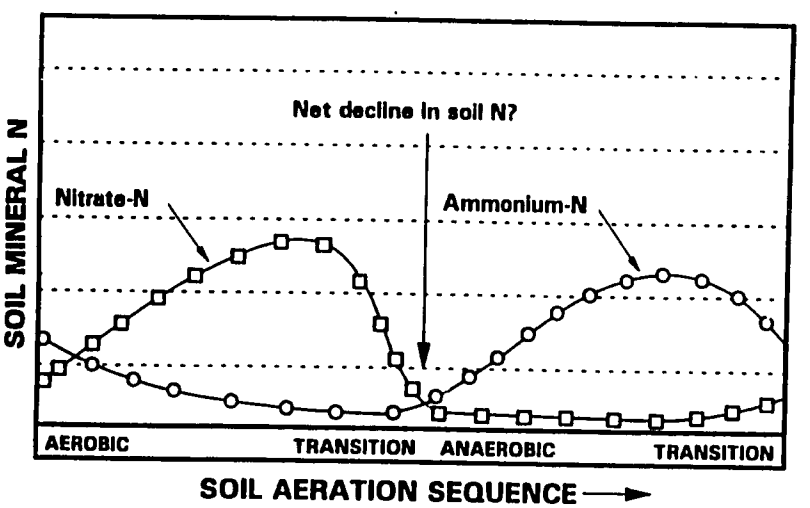


Figure 1. Conceptualization of N dynamics in lowland rice-based cropping systems

Collaboration to Maximize BNF in IITA's Legume Breeding Program

NifTAL and the International Institute of Tropical Agriculture (IITA) in Nigeria are collaborating on the study of the symbiosis between promiscuously nodulating soybean genotypes and their affiliated *Bradyrhizobium* spp.

Under the supervision of soybean breeder Dr. Ken Dashiell in IITA's Grain Legume Improvement Program, NifTAL scientist Robert Abaidoo will examine the indigenous bradyrhizobia which nodulate the IITA lines. The goal of the project is to determine the potential of indigenous bradyrhizobia to provide sufficient nodulation and nitrogen fixation to meet the yield potential of the "promiscuously nodulating" lines of soybean selected at IITA.

This two year program was initiated in September, 1991, and will involve extensive sampling of bradyrhizobial populations from several environments and field inoculation trials comparing the indigenous population with introduced *B. japonicum.*

Collaboration with MARDI to Identify Acid Tolerant Leucaena Lines and Rhizobia

Although *Leucaena leucocephala* is a valuable nitrogen fixing tree for soil stabilization and agroforestry situations, it is extremely sensitive to the acid soil conditions prevalent in the humid tropics. In January, 1989, NifTAL received a three year USAID contract to develop acid tolerant *Leucaena leucocephala* lines to extend the environmental range of this useful tree. This breeding work was subcontracted to the Malaysian Agricultural Research Development Institute (MARDI), while NifTAL identified an effective, acid tolerant rhizobial symbiont for the improved varieties.

Effective *Leucaena*-rhizobia combinations adapted to Malaysian acid soils will be useful contributions to the country's agriculture. Adapted varieties of *Leucaena* will provide Malaysian farmers with high protein forage as well as high protein leaf meal for chickens and pigs. The trees can also serve as a source of wood for construction, paper pulp, and fuel for drying tobacco and cooking purposes. This research will be of interest to other tropical countries with substantial areas of acid soils.

On-Farm Trials in the Philippines

Competitive grants from the USAID PSTC have extended NifTAL's ability to conduct collaborative research in the Philippines. The Philippine Council for Agriculture and Resources Research and Development (PCARRD), the National Tobacco Administration, Ilagan Experiment Station, the University of Philippines at Los Banos (UPLB), and NifTAL are working together conducting on-farm trials to determine the economic benefits of using starter nitrogen in mungbean crops.

In another collaborative project with PCARRD, Philippine Tobacco Authority, Tarlac College of Agriculture, and UPLB, a series of experiments were designed to evaluate the impact of rice crop duration on the yield and BNF of sequentially cropped legumes. Results indicate that low input technologies such as appropriate varietal selection of the rice and legume inoculation can increase incomes in these cropping systems. Details on these projects are in the Research section of this report.

Technical Assistance and Technology Transfer

NifTAL's Activities Overseas

NifTAL provides technical assistance to developing countries by responding to requests for training, collaborative research, consultancies, and material and informational resources. The following are highlights of NifTAL's overseas activities. Details are provided in other sections of this report.

Bhutan

BNFRC's Dr. Nantakorn Boonkerd visited Bhutan in July, 1990, to provide technical assistance in inoculant production.

Haiti

NifTAL continues to provide support and technical assistance to Haiti's commercial inoculant production facility, Medicom, Inc. NifTAL inoculum specialist, Joe Rourke, helped to set up the laboratory in 1989, and has since provided rhizobial strains, business contacts, and references.

India

Indian and U.S. scientists collaborated on a scientific exchange and joint research from 1984-1991. The Science and Technology Initiative Workshop for 1991 was held on Maui, Hawaii from September 16-18. Drs. Paul Singleton and Padma Somasegaran were involved in the STI, and presented a paper on their research findings.

Indonesia

To address the concern of technology transfer from the extension specialists to the farmer, NifTAL and the BNFRC, Thailand, conducted a training course from March 25 - April 6, 1990, for Indonesian extension specialists. NifTAL personnel Drs. Paul Singleton and Padma Somasegaran and Ms. Princess Ferguson were course instructors.

Malaysia

NifTAL received a three-year USAID contract to develop acid tolerant *Leucaena leucocephala* lines to extend the environmental range of this useful tree. The breeding work was subcontracted to the Malaysian Agricultural Research Development Institute (MARDI). NifTAL scientist, Dr. Padma Somasegaran visited this institute in July, 1991, to assist local researchers in designing experiments to assess the nitrogen fixing ability of their plant selections.

Morocco

For the first time in Northern Africa, NifTAL offered a training course in "Techniques in Rhizobiology of Pasture and Forage Legumes" from April 22 - May 5, 1990. Mr. Heinz Hoben was an instructor/coordinator for the workshop.

Nepal

In December, 1990, NifTAL staff Dr. Paul Singleton responded to USAID's request to conduct a preliminary evaluation of the BNF capabilities in this country and to identify constraints to delivery of the technology.

Nigeria

NifTAL and the International Institute of Tropical Agriculture (IITA) are collaborating on the study of the symbiosis between promiscuously nodulating soybean genotypes and their affiliated *Bradyrhizobium* spp. NifTAL has stationed a researcher, Mr. Robert Abaidoo, at IITA, and Dr. Harold Keyser visited IITA in September, 1990, to participate in the research planning. In another project, BNFRC's Dr. Nantakorn Boonkerd has provided technical assistance in inoculant production to the Anambra State University of Enugu in April 1990.

Philippines

On-farm trials are being conducted in Isabella and Ilocos Norte to determine the economic value of applying starter N to legume crops. In addition, NifTAL agronomist Dr. Thomas George is stationed in the Philippines at the International Rice Research Institute to study nitrogen economies of paddy-rice systems.

Thailand

The NifTAL BNF Resource Center, Thailand, continues as an outreach support for S.E. Asia. The Khon Kaen University, Thailand, and NifTAL are collaborating on a PSTC project to enhance ecologically based modes of legume response to inoculation.

Togo

In an agreement with the International Fertilizer Development Center (IFDC) in Lome, Togo, NifTAL will test soils from sites along the humid-savannah-Sahel transect for effectiveness of native populations of rhizobia. NifTAL will supply inoculants and IFDC is considering a regional training course based on NifTAL's training manual, *Applied BNF Technology: A Practical Guide for Extension Specialists.* This agreement was reached following a visit by Dr. Harold Keyser in September, 1991.

Uganda

Through the MFAD Project, NifTAL recently established an inoculant production facility at Makerere University, Kampala. A needs assessment was conducted in 1989 by NifTAL's Dr. Paul Singleton. In September, 1990, Joe Rourke visited Uganda to develop a rhizobium inoculum production unit and to train laboratory personnel. In December, 1990, Harold Keyser provided technical coordination and assistance, and conducted training of extension personnel.

Zambia

A NifTAL installed laboratory and inoculant production facility at the Mt. Makulu Research Station is now supplying a higher quality inoculum in greater quantities. NifTAL continues to provide technical support. During a visit from Joe Rourke in July, 1991, two 100 L NifTAL designed fementation vessels were assembled and tested.

Inoculant Production Facility Established in Uganda

In a cooperative agreement with USAID S&T/AGR, Washington D.C., Ohio State University, and the MFAD S&T project-Uganda, the NifTAL Project participated in establishing inoculum production capabilities for legumes at Makerere University, Faculty of Agriculture and Forestry, Department of Soil Science, Kampala, Uganda.

In late 1989, Paul Singleton of NifTAL conducted a needs assessment in Uganda. This assessment included a soil survey, the determination of the potential of soybean varieties to nodulate spontaneously with native soil rhizobia, and the search for a useful peat source. He presented a workshop on BNF technology, transferred a culture collection of rhizobia to Makerere University for grain and tree legumes, evaluated their present resources and planned development of the facilities. At that time Dr. Singleton determined that farmers could expect a 500 kg/ha yield increase with inoculation and an increase of 1000 kg/ha with inoculation and the application of 30 kg/ha P_2O_5 in the P deficient soils north of Kampala.

In September, 1990, NifTAL inoculum production specialist, Joseph Rourke, visited Uganda to develop a legume inoculant production unit, train laboratory personnel, and locate a suitable source of indigenous peat and test its suitability as an inoculum carrier.

In December, 1990, NifTAL microbiologist, Dr. Harold Keyser, visited Uganda for the purpose of providing technical coordination and assisting in the teaching of a course, "Biological Nitrogen Fixation Technology Workshop for Extension Specialists".

Renovations to the Makerere facilities were coordinated with Julius Zake, Chairman of the Department, M.A. Bekunda, Head of Inoculum Production, and Charles Nkwine, Supervisor of the lab. Technical staff received training in microbiological procedures and quality control technique.

A renovated facility at the Kabanyolo Research Center houses two NifTAL designed fermentation pressure vessels. Located at Kabale, Uganda are natural peat deposits which were tested and found satisfactory. (See article on **Quality Assurance & Materials Testing**). Indigenous calcium carbonate is used to neutralize the peat. Three to five years of laboratory supplies and a collection of NifTAL Rhizobial strains complete Uganda's needs to facilitate production of inoculum for grain, pasture, and tree species.

Inoculant Production Capacity Potential Increases in Zambia

Since 1984, NifTAL has assumed a pivotal role in establishing and maintaining inoculant production capabilities in Zambia. Through a sub-contract from the University of Illinois' ZAMARE Project, NifTAL installed laboratory and production facilities at the Mt. Makulu Research Station. During this period, NifTAL stationed a microbiologist to conduct research, provided an inoculant specialist for three planting seasons, and trained local personnel. While the operations at Mt. Makulu are now becoming self-sufficient and semi-commercial, NifTAL has continued to provide technical support.

Because responses to inoculation have frequently been observed in Zambia, the use of inoculants for legume crops has been increasing rapidly. Inoculum demand has challenged the facility's ability to keep pace. An estimated 8000 ha of soybean were inoculated the first year. There was a 300% increase in 1985, followed by annual increases to 40,000 ha of soybean receiving inoculation.

The facility originally housed glass flask fermentors and 20 L dilution vessels. Following a week long visit in July, 1991, by NifTAL Inoculant Production Specialist, Joe Rourke, two NifTAL-designed 100 L fermentation pressure vessels were assembled and tested at the Mt. Makulu BNF laboratory. These fermentors are sterilized by heating with propane burners for one hour, and cooled by stainless steel cold water coils. They are then ready to be inoculated with a starter culture, all within a day. Turn around times for fermentor harvests average 10 working days. These fermentors are capable of providing both the quantity and high quality of inoculants the growing Zambian market demands.

Technical Support of Commercial Inoculant Production in Haiti

A consortium of various private voluntary organizations, including CARE and the Pan American Development Foundation, are leading an effort to combat the massive deforestation on Haiti. They are planting thousands of trees, primarily nitrogen fixing legumes, to stabilize eroding hillsides and conserve soil resources. These agencies depend on Medicom, Inc., a commercial inoculant production facility in Port-au-Prince, to provide their legume inoculants.

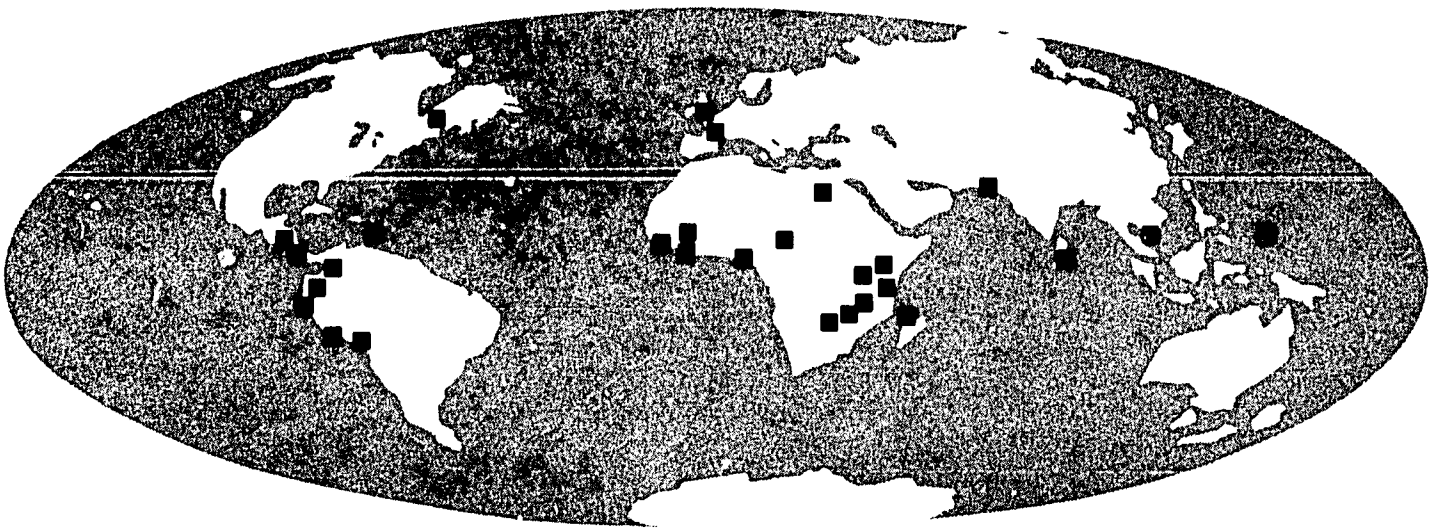
Prior to the establishment of Medicom's inoculant production facilities through the Agroforestry Outreach Project (AID/Haiti), NifTAL provided the tree inoculants for the reforestation efforts. In July, 1989, NifTAL microbiologist Harold Keyser visited Haiti and assessed Medicom's laboratory facilities. In September of that year, Ms. Muriel Bouchareau, microbiologist and owner of Medicom, spent two weeks at NifTAL. She received intensive training in rhizobiology, and worked with NifTAL staff to design an inoculant production package to meet her company's needs. NifTAL's inoculant production specialist, Joseph Rourke, then visited Medicom to assemble and install specialized equipment and to provide on-site training. Before the year's end, the first batches of inoculum were produced.

NifTAL continues to provide technical support for Medicom's legume inoculant production in Haiti. Support includes inoculant quality control assessment and provision of recommended strains of rhizobia, based upon on-going research on tree legumes at NifTAL. Medicom's success illustrates the potential for NifTAL to assist entrepreneurship in developing countries.

NifTAL Effort is Good for U.S. Business

An inoculant production process developed by NifTAL is being used by a U.S. legume inoculant company. This process involves the use of diluted rhizobial broth and sterile carriers. This technology saves time, overhead, and material costs without sacrificing the quality of the product. Although it was developed for agribusiness use in A.I.D. assisted countries, Titre Inc. of Montana has adapted the NifTAL process and has been producing inoculants for three years. The company currently produces nearly three tons of legume inoculants per month, and over 60% of the production is exported. This same production process used by Titre, Inc. has also been adopted for use by private inoculant producers in Morocco, Thailand, Haiti, and Indonesia.

Material and Informational Services



Research material distribution in 1990

Quality Assurance and Materials Testing

The success of inoculant production facilities depends on both the high technical standards of the personnel and the quality of the components. Product and materials testing is the most important process for monitoring and maintaining high quality production. This service is provided by NifTAL to scientists and inoculant producers worldwide.

The efficiency and economic viability of inoculant production enterprises are related to their access to suitable carrier materials. The use of inexpensive, indigenous materials as carriers can reduce production costs significantly. NifTAL analyzed a variety of materials considered for use as inoculant carriers.

The materials varied from forest soil mixed with charcoal (Philippines), raw indigenous peat (Zambia, Uganda, Thailand), to high quality commercial peat (Australia, USA). NifTAL evaluated the carbon and nitrogen contents of the materials, their relative distribution of particle size, and their ability to sustain rhizobial populations over time. **Table 1** shows the survival of rhizobia in indigenous Ugandan peat compared to standard commercial peat from the U.S. Our evaluation showed that survival in the Ugandan material was comparable to the U.S. commercial carrier, and suitable for locally produced inoculants.

Table 1. Survival of rhizobium strains in two non-sterile carriers. Samples incubated at 26-28° C.

	Days	Soybean	Bush bean	Leucaena
Uganda	10	2.1E+09	1.8E+09	1.2E+10
	30	1.2E+09	2.0E+08	4.0E+09
	60	4.2E+07	1.4E+08	1.9E+08
	90	2.0E+07	1.1E+07	6.3E+07
USA	10	1.9E+09	8.0E+09	8.3E+09
	30	1.2E+09	2.1E+09	5.3E+09
	60	4.2E+07	9.0E+07	1.0E+09
	90	2.4E+07	2.3E+07	7.9E+08

Information Dissemination

NifTAL's Communication Section is responsible for providing information about the Project and about specific technical or research topics. We fill hundreds of requests from NifTAL's wide variety of publications. We also provide citations and contacts for authors of current BNF articles from an array of American and international journals. NifTAL's Continuing Bibliography, an insert to the BNF Bulletin, is a resource for developing country scientists who do not have access to informational data bases and journals.

The Communications Section also taps the expertise of NifTAL scientists to generate informational brochures, bulletins, texts and manuals. We also distribute and sell NifTAL developed computer software for decision support. Some of the services and informational products disseminated by the Communications Department are listed below:

Journal Articles: NifTAL researchers publish their works in well respected scientific journals and books. These articles and book chapters are constantly in request. Abstracts of current NifTAL publications are presented in the Appendix.

BNF Bulletin: This informational newsletter is published twice a year to inform readers from over 100 countries of BNF activities, training, scientific research, commercial products and technologies. It is mailed to approximately 1700 individuals and institutions who share an interest in BNF.

Bibliography: The Continuing Bibliography listing hundreds of current BNF citations is included as an insert in the BNF Bulletin. A computerized citation service keeps the information current.

Books and Manuals: We sell several NifTAL developed books and manuals, or distribute them as part of training packages. Titles include the following:

Methods in Legume Rhizobium Technology: A training manual for basic rhizobiology, currently undergoing revision to include advanced techniques.

Legume Inoculants and Their Use: An excellent handbook especially useful to extension workers.

Dynamics of Soil Organic Matter in Tropical Ecosystems: This state-of-the-art book was produced by NifTAL following a conference of top experts in this field.

Applied BNF Technology: A Practical Guide for Extension Specialists: This modular training program features a packet of training materials, practical exercises, and demonstrations to "train trainers". Details are in the Research and Development section of this report.

Computer Software:

RESPONSE: is derived from NifTAL's ecological studies on the performance of rhizobial strains released into various agro-ecosystems. It is useful to predict the inoculation response of crops based on measurable environmental parameters.

ACCLAIM: provides information on NifTAL's germplasm collection as well as specific protocols for seed preparation and inoculation.

MPNES: Most-Probable-Number Enumeration System calculates and tests the reliability of results from plant infection tests used to estimate rhizobial populations. This program is used by commercial inoculant manufacturers and quality control programs worldwide.

FAIME: Financial Analyses for Inoculant Manufacturing Enterprises provides realistic economic projections for inoculant manufacturers. This program is featured in the R&D section of this report.

Miscellaneous Publications: These include training brochures, annual reports, directories, video "The Missing Link" produced in 1989, and Illustrated Concepts in Agricultural Biotechnology.

Germplasm, Inoculants, and Antisera Services

NifTAL continued to provide rhizobial strains, inoculants, and antisera to BNF researchers worldwide. Requests for rhizobial strains and inoculants effective on tree legumes were again popular. The following table details the number of requests for these materials from January 1, 1990, to September 30, 1991.

Material	Countries	Requests	Total sent
Germplasm 1990	26	62	621 strains
Germplasm 1991	20	44	419 strains
Inoculants 1990	20	113	673 packets
Inoculants 1991	12	70	250 packets
Antisera 1990-91	8	12	164 ml

Training Courses and Workshops

Training Course for Indonesian Extension Specialists

In 1989, NifTAL 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 Agricultural Products. We found that despite the availability of high quality inoculants and Indonesia's institutional capacity to conduct BNF research, the use of inoculants by farmers was low. The assessment concluded that the constraint to inoculant use was the lack of awareness of the benefits of BNF by farmers and extension personnel.

Dr. Paul Singleton discusses field demonstration with Indonesian extension specialists during a training course at Khon Kaen, Thailand.

To address this weak link in technology transfer, NifTAL and the BNFRC in Bangkok conducted a training course in applied BNF technology for Indonesian extension specialists from March 28-April 5, 1990, in Khon Kaen, Thailand. Fifteen extension specialists and BNF researchers from Indonesia participated. Course instructors included; NifTAL's head of research Dr. Paul Singleton; microbiologist Dr. Padma Somasegaran; Information Officer Ms. Princess Ferguson; Dr. Nantakorn Boonkerd, Director of the BNFRC; Dr. Saroso Sindhoesarojo, Director of the Secondary Food Crops Development Project, Indonesia; Dr. Mintarsih Adimihardja, Dean of the Faculty of Agriculture, University of Lampung, Indonesia; Dr. Brian Hilton, Communications for Technology Transfer in Agriculture, Indonesia; Mr. Chirasuk Arunsri and Mr. Precha Wadisirisuk, researchers for the Department of Agriculture, Thailand.

The extension manual developed for the course, *Applied BNF Technology: A Practical Guide for Extension Specialists*, is a modular package, with each module containing

Trainees were exposed to laboratory procedures using BNF.

technical information, instructions for demonstrations, prototypes for handouts, and case studies for self evaluation and problem solving. This publication was prepared and produced by the scientific, training, and communication staff of the NifTAL Project, and was translated into Indonesian.

The participants evaluated BNF field experiments and environmental interactions, learned proper procedures for handling inoculants, and also practiced educational techniques to develop farmer awareness of inoculant products. This type of training of extension specialists is a vital link between NifTAL's scientific research on BNF technology and its actual benefit to farmers.

Trainees examine root nodulation.

Indo-U.S. STI Workshop

The Indo-U.S. Science and Technology Initiative (STI) was formed in 1984 following an agreement between U.S. President Ronald Regan and India's Prime Minister, Indira Gandhi. This agreement initiated a collaborative effort between India and the United States for scientific exchange and joint research. NifTAL, a participant in this program since its inception, hosted the fourth and final STI Workshop on Maui, Hawaii, from September 16-19, 1991. There the participants met to summarize their research and discuss the potential for future collaboration.

India's main purpose in this agricultural research program was to expand yield, while the U.S. targeted soil and environment preservation. Reforestation and efficient use of nitrogen have both been subjects of ongoing research. The U.S. agricultural research projects fell into three categories: biological nitrogen fixation (BNF), nitrogen fertilizer efficiency (NFE), and biomass research.

Researchers at the NifTAL Project concentrated on BNF, and used a broad approach to reveal basic principles governing the effective use of BNF for mankind's benefit. One determination is that benefits from inoculating legumes are inextricably linked to environmental factors affecting both the host plant and its symbiont. Our research was directed at elucidating how or which processes of the symbiosis should be manipulated by management inputs to enhance BNF. We evaluated the effects of various management inputs and environmental factors on BNF and interstrain competition, the role of cropping systems on the ecology of native rhizobia, and the regulation of nodulation by the host. Other aspects of NifTAL's Indo-U.S. sponsored research addressed the availability, cost, and quality control of inoculants.

NifTAL staff Drs. Paul Singleton and Padma Somasegaran joined 25 scientists from India and the U.S. at the workshop. The collaborators presented papers on their completed projects and joined these efforts into a final publication. The participants agreed that the Indo-U.S. STI program has provided valuable opportunities, both for scientific accomplishments as well as for professional interaction.

Dr. Haruyoshi Ikawa of the University of Hawaii's Department of Agronomy and Soils shows Indo-US STI workshop participants the soil structure at one of the Maui Net sites.

Mubende Workshop for Extension Specialists

Trainees at Uganda workshop learn simple techniques to inoculate seeds.

A "Biological Nitrogen Fixation Technology Workshop for Extension Specialists" was held at Mubende District Farm Institute, Mityana, Uganda from November 26-30, 1990. The course was presented through the BNF/Legume Management Outreach Pilot Project (S&T/AGR), a consortium of PVOs and the NifTAL Project.

The workshop was sponsored by Agricultural Cooperatives Development International, the Uganda Cooperative Alliance and the BNF/Legume Management Outreach Pilot Project (BNF/LM OPP). It was coordinated by Makerere University Faculty of Agriculture and Uganda Cooperative Central Union.

The 24 participants were extension specialists from PVO, NGO and the Ministry of Agriculture. The workshop focused on the applied aspects of BNF technology, emphasizing inoculant use and handling, trouble-shooting problems with BNF, and techniques for teaching inoculant usage to farmers. The course format was based on NifTAL's training manual *Applied BNF Technology: A Practical Guide for Extension Specialists.* Dr. Harold Keyser from NifTAL provided technical coordination and assisted in teaching course material. Other instructors included Mr. Charles Nkwine (Makere University), Dr. Gadi Gumisiriza (Ministry of Agriculture), Ms. Marion van Schaik (BNF/LM OPP), and Kwesiga Rweenda (CARE-Uganda).

Through the MFAD Project, NifTAL recently established an inoculant production facility at Makerere University in Kampala. This training course complemented the various USAID funded efforts to provide BNF awareness, technical expertise, extension training, and inoculant production capabilities to Uganda.

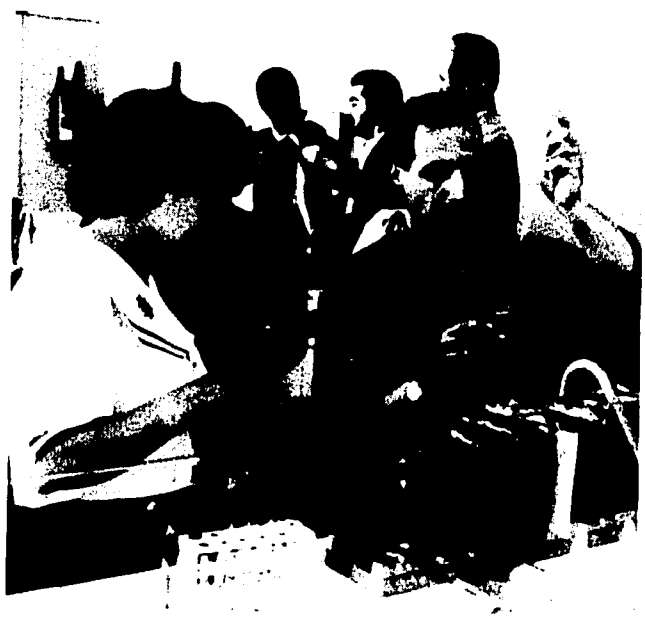
(See **Inoculum Production Facility Established**, also in this section)

Training in BNF for North African Scientists

Scientists representing five North African countries were selected to participate in a training course entitled "Techniques in Rhizobiology of Pasture and Forage Legumes," held at the University of Moulay Ismael, Meknes, Morocco from April 22 - May 5, 1990. This training course was the first offered by NifTAL in the North African region. There is a need to improve pastures by promoting the use of legumes in this area. The benefits of BNF to pasture systems may help to increase the load capacity for animal grazing in the dry North African and West Asian Mediterranean regions.

The course was a collaborative effort between NifTAL Project, the Food and Agricultural Organization of the United Nations (FAO), University of Moulay Ismael, and the International Center for Agricultural Research in the Dry Areas (ICARDA) in Aleppo, Syria. Dr. Luis Materon of ICARDA, Dr. Mohammed Ismaeli of University of Moulay Ismael, and Mr. H.J. Hoben of NifTAL coordinated the course and were the principle instructors.

ICARDA and FAO supported the 14 microbiologists and agronomists from Algeria, Egypt, Lybia, Syria, and Morocco who participated in the course. These scientists received intensive training in basic rhizobiology and in the use and production of rhizobial inoculants. Their training will strengthen the capabilities of government institutes within these North African countries to develop and implement BNF programs.



Trainees from North African regions practice methods used in the quality control of inoculants.

Encore for " Modern Methods for the Study of Rhizobia"

After an enthusiastic response to the NifTAL training course "Modern Methods for the Study of Rhizobia" first held in 1989, the course was again offered from June 3-28, 1991 in the Rhizobium Building of the Thai Department of Agriculture, Bangkhen, Thailand.

Fourteen scientists from nine countries, Bangladesh, the Peoples Republic of China, Malaysia, Uganda, Vietnam, Pakistan, the Philippines, Sri Lanka, and the USA, received training. Areas of emphasis were the evaluation and assessment of nitrogen fixation, inoculant production and quality control, serological techniques for strain identification, and genetic technologies.

The main instructors and lecturers were Dr. N. Boonkerd, local organizer and Director of NifTAL's BNF Resource Center; Dr. P. Somasegaran, course director and NifTAL microbiologist; Mr. H. Hoben, international organizer and NifTAL training coordinator; Dr. S. Danso, IAEA scientist; and Dr. D. Herridge, research scientist of NSW Agriculture and Fisheries, Australia.

This course was co-sponsored by the International Atomic Energy Agency (IAEA), the BNF Resource Center, the Thai Department of Agriculture and the NifTAL Project. This collaborative effort helped research and production oriented scientists upgrade their knowledge and practical experience in legume/rhizobia based technology.

Modeling N-Fixation for Grain Legume Crops

A three day workshop was held in Maui, Hawaii from March 15-17, 1990, on "Modeling N-fixation for Grain Legume Crops." The workshop was organized and sponsored by IBSNAT, University of Florida, University of Georgia, University of Hawaii, and the NifTAL Project.

The workshop was a collaborative effort to integrate a symbiotic N_2 fixation subroutine into the process-level crop growth simulation model, SOYGRO.

Dr. Padma Somasegaran (center) instructs trainees in the Modern Methods course.

Visiting Scientists

Dr. Nesime Cebel

Dr. Cebel is a visiting scientist from the Soil and Fertilizer Research Institute in Ankara, Turkey. She earned her doctorate degree from Ankara University, Faculty of Agriculture, and is currently researching the development and characterization of strain/group specific DNA probes for the identification of chickpea nodulating rhizobia.

With support from the Organization for Economic Cooperation and Development (OECD), Dr. Cebel has been researching at NifTAL for the past six months, and will continue her work here until early 1992.

Dr. Andreas Wolff

Dr. Wolff researched at NifTAL in the spring of 1991, with a special interest in the correlation between the amount of tannin and the number of root nodules. Dr. Wolff is with the Department of Biology at the University of Marburg, Germany. He hopes to return to NifTAL to continue his work, and plans to set up field experiments to explore the effects of elevation and stress conditions on inoculation.

Janez Hacin

Janez Hacin, a researcher from the University of Ljubljana, Ljubljana, Yugoslavia, is working at NifTAL in BNF research. Of primary interest to Mr. Hacin is elucidation of possible mechanisms involved in host control of nodulation in soybeans.

Mr. Hacin is a research fellow supported by the Organization for Economic Co-operation and Development (OECD) in their Co-operative Research Project on Food Production and Preservation Program. His work at NifTAL is to be applied towards his Ph.D. degree with completion scheduled forJanuary, 1992. He will then be defending his thesis in February at the University of Ljubljana. His plans for the future include further research on BNF which he hopes will be applied to relevant problems in Europe. He also plans to teach at the University in Ljubljana, Slovenia.

Remi Rakotondradona

Dr. Rakotondradona is a lecturer/researcher, and Chef de Service des Sciences at the University of Antananarivo, Madagascar. He received his Ph.D. from the Department of Plant Sciences at Washington State University.

Dr. Rakotondradona's work at NifTAL is sponsored by the African American Institute, while he is being trained in applied biological nitrogen fixation technologies. He will be responsible for promoting BNF extension in Madagascar upon his return. During his stay at NifTAL, Dr. Rakotondradona is also conducting research on the promiscuously nodulating soybeans being developed for Africa, and learning critical evaluation of the benefits of BNF in the field.

Recent NifTAL Graduate Students

Maria Luz Caces George

Dr. George completed her degree work in the fall, 1990, under the direction of Dr. Francoise Robert, Department of Microbiology, University of Hawaii. Her dissertation research concerned the relationship between competitiveness of strains of *Rhizobium leguminosarum* bv. *phaseoli* and their ability to suppress nodulation across split-roots of beans.

Dr. George is currently conducting postdoctoral research with Dr. Dulal Borthakur, Biotechnology Program, University of Hawaii.

Paul Woomer

Dr. Woomer completed his Ph.D. degree in the spring of 1990 with a thesis entitled "Predicting the Abundance of Indigenous and the Persistence of Introduced Rhizobia in Tropical Soil." His work was supported by USAID/NifTAL, and by a grant from NSF/Ecology.

While with NifTAL, Dr. Woomer coauthored the computer software program, *Most Probable Number Enumeration System*, useful in the enumeration of microorganisms in soils, foods, and water. Dr. Woomer completed his Ph.D. degree in the spring of 1990, and is currently employed by the Tropical Soil Biology and Fertility (TSBF) Program, and is stationed at the UNESCO Regional Office for Africa, Nairobi, Kenya.

Janice Thies

Dr. Thies completed her Ph.D. in the summer, 1990, with the support of USAID/NifTAL and a grant from NSF/Ecology. Her thesis, *Modeling Ecological Determinants of the Symbiotic Performance of Introduced Rhizobia in Tropical Soils*, was published in Applied and Environmental Microbiology. Dr. Thies' work is a foundation for development of ways to predict legume response to rhizobial inoculation by using indices of indigenous rhizobial populations and the nitrogen status of the soil.

From March 15-17, 1990, Dr. Thies hosted an IBSNAT/NifTAL Collaborative workshop on integrating a symbiotic N_2 fixation subroutine into the process-level crop growth simulation model, SOYGRO.

Dr. Thies presented an oral report at the North American symbiotic Nitrogen Fixation Conference on Environmental Effects on Rhizobial Interstrain Competition for Nodule Occupancy in August, 1991, and is currently working as a postdoctoral fellow with Dr. David Myrold at the Department of Crop and Soil Science, Oregon State University, Corvallis.

Graduate Students

Marcelo Bartolini

Marcelo Bartolini, a Masters in Business Administration major at the University of Hawaii, holds a M.Sc. in Food Science and Technology from the University of Georgia and a B.S. in Agricultural Engineering from the Universidade Nacional de Cuyo in Mendoza.

Mr. Bartolini is presently working with Dr. Paul Singleton and Joseph Rourke at NifTAL on FAIME, a computer model for inoculant production cost analysis. The computer model is expected to be applicable to any set of circumstances in any country, and will give potential producers a method to determine the price that is necessary to charge for inoculants in order to have a profitable business.

In June, 1991, Mr. Bartolini and Joseph Rourke, NifTAL's inoculant production coordinator, visited Rhizobium production facilities in Australia, Indonesia, Thailand and the Philippines. The purpose of the visits was to determine production costs and plant and machinery specifications that would allow them to make recommendations for the facilities and suggest alternative actions.

In 1990, Mr. Bartolini published a paper on Molecular characteristics of Pectins from Enzyme Tested Apple Juices in the Journal of Food Science SS(2):564-5. He will graduate from the University of Hawaii in May, 1992.

Ph.D. candidate Surya Tewari examines plants in her field experiment at NifTAL's Kuiaha site.

Moradeke Fadare

Moradeke Fadare is currently a graduate student at the University of Hawaii, Manoa, doing her research work at NifTAL on rhizobial requirement of legumes used in the Alleyu Farming Network for Tropical Africa (AFNETA).

Ms. Fadare is from Nigeria and sponsored by a grant from IITA, Ibadan, Nigeria.

Veronique T. Lambert

Veronique Lambert is a graduate student at the University of Hawaii, Manoa, doing research work at the NifTAL Project. Prior to enrolling in graduate studies, Ms. Lambert worked for the Guyana Sugar Corporation evaluating soybean cultivars and management practices for the crop diversification program. Ms. Lambert has not yet chosen her research topic, but will continue research with NifTAL and will graduate in 1993.

Surya Tewari

Surya Tewari is researching the influence of soil nitrogen on early soybean growth and development with a view to improving crop modeling techniques based on collected data. While doing her research at NifTAL, Ms. Tewari is taking course work at the University of Hawaii, Manoa, and working toward her Ph.D.

Ms. Tewari is from the twin island Republic of Trinidad and Tobago, and earned a B.Sc. in General Agriculture from the University of the West Indies, St. Augustine, Trinidad, and her M.Sc. degree at Wye College, University of London.

Bruce Linquist

Bruce Linquist is presently working on his Ph.D. degree in Ecology at the University of California, Davis. He earned a B.S. degree from Texas A&M at College Station, Texas, and a M.S. degree in International Agricultural Development.

Bruce is working at NifTAL on phosphorus uptake in legumes as affected by root length, mycorrhizal fungi, and symbiosis with rhizobia.

Research and Development

The extraordinary nitrogen fixing partnership between legumes and rhizobia has intrigued scientists since its discovery a century ago. The potential benefits of BNF have motivated an intense and diverse research and development effort to understand the symbiosis and to exploit it for mankind. There are few aspects of agriculture that are as economically important and intellectually challenging as this symbiosis.

Environmental, biological, and methodological constraints limit BNF. NifTAL's research portfolio reflects an integrated, multidisciplinary approach to removing these constraints. We and our collaborators have joined our resources for both short and long range projects. These share common justifications - they address general principles of the symbioses and have wide applicability.

The development section of NifTAL's portfolio is product oriented. We have synthesized and integrated our research, outreach, and communications efforts to provide tools to facilitate BNF technology transfer to developing countries. During this reporting period we concentrated on cost efficient methods for inoculant production, computer programs for decision support for the inoculant industry, a training package for extension specialists, and simplified techniques for researchers.

NifTAL's research and development program has been organized into four general objectives. These objectives are; environmental constraints to enhanced BNF, host-rhizobia interactions, methodologies for understanding BNF, and tools for development. Ongoing projects are detailed in this section. Abstracts of published works are provided in the Appendix.

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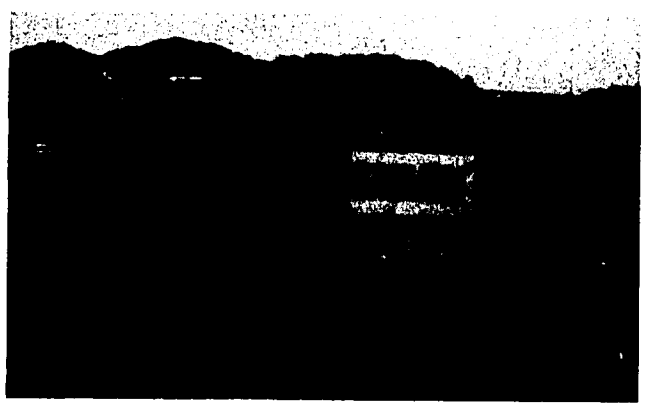
Environmental Constraints to Enhanced BNF:

The legume nitrogen fixing system can only be as effective as the environment allows it to be. Environmental stresses which affect any of the complex plant and microbe processes of the symbiosis results in reduced BNF. We are developing management strategies to maximize BNF within the context of environmental conditions and soil stresses common to agro-ecosystems of the tropics. These conditions include soil phosphorus deficiencies, aerobic-anaerobic soil fluxes, and legume-grain cropping relays. We also continued to build upon our previous work linking inoculation responses to environmental parameters, and testing and validating our predictive models of inoculation response.

Conservation and Use of Soil and Atmospheric Nitrogen through Legumes in Lowland Rice-based Cropping Systems

NifTAL, IRRI, and IFDC (see **Networking and Collaboration** Section) have initiated a joint study to describe the nitrogen dynamics of lowland rice-based cropping systems, and the effect of legume BNF in fallow-legume-rice and legume-fallow-rice sequences. The goal of this collaborative research is to identify soil and plant management strategies to maximize production while conserving soil N resources. Before there is sufficient water to plant lowland rice, soils are intermittently wet and dry. These conditions promote the accumulation of NO_3 in the soil profile. Most



Legume, weed, and weed free treatments in the wet-dry transition period.

of this NO_3 is lost through denitrification and leaching upon soil flooding for rice production. Management strategies need to be designed that retain soil N resources and add additional N to the system through BNF. Results from preliminary trials show potential legume-BNF contributions of more than 150 kg N ha^{-1}. Because much of the world's population in the developing tropics depend on lowland rice cropping systems, the integration of nitrogen fixing legumes into these systems may have significant impacts in raising farm income through increased productivity and reduced input cost. Management strategies developed from the research will also reduce the contamination of groundwater resources with No_3.

Field studies have been established in the Philippines using soil/crop management factors to vary available soil nitrogen levels and legume BNF. Researchers continuously monitor nitrate (NO_3) and ammonium (NH_4) in different soil layers. BNF is measured by ^{15}N techniques. In addition, ^{15}N labeled legume residues grown in situ will enable the researchers to determine the fate of legume nitrogen.

Preliminary data from the first-year experiments indicate the potential for soil nitrate accumulation and loss, and the role of legumes and weeds to conserve and efficiently use nitrogen in lowland rice-based cropping systems. Nitrate nitrogen in the 0 to 60 cm soil layer at the end of a dry season fallow in April ranged from 14 to 108 kg N ha^{-1}, depending upon weed growth and tillage (**Table 2**). Soil nitrate decreased during the dry to wet transition period due to plant uptake and nitrogen losses (**Tables 2&3**). Uptake of soil nitrogen during the dry to wet transition period was 31 to 35 kg N ha^{-1} by weeds, 33 to 66 kg N ha^{-1} by mungbean, and 46 to 97 kg N ha^{-1} by Sesbania (**Table 4**). The amounts and proportions of nitrogen fixed by the legumes decreased with increasing soil nitrate levels at planting (**Table 4**). Sesbania planted to plots maintained as weedy fallow or weed-free fallow derived 154 kg N ha^{-1} from the atmosphere. Nitrogen available for recycling to the rice crop through sesbania, mungbean and weed residues in the tilled treatment were 222, 77 and 35 kg N ha^{-1}, respectively. Nitrate not used by plants during the transition period was lost. Less than 10 kg nitrate-nitrogen ha^{-1} remained 1 wk after flooding for rice production. Data on the magnitudes of nitrate loss, the fate of legume nitrogen (see 'weed free treatment' in **Table 3**) and the nitrogen nutrition and yield of subsequent rice crop are presently being generated. These data indicate that low input management techniques can be devised that will retain soil N resources and enhance N available in lowland rice-based cropping systems through legume BNF.

Table 2 . Soil nitrate as influenced by fallow management during the dry season and dry to wet transition period in lowland rice.

Fallow management*	Soil nitrate (0-60 cm)						
	1 Feb	26 Feb	22 Mar	26 Apr	23 May	13 Jul	27 Jul
	kg NO_3-N ha^{-1}						
Weedy	28	26	13	14	27	12	8
Weed free	28	48	64	73	59	32	9
Tilled-wet/dry**	28	43	81	108	100	56	10

*Management was initiated on 1 February after tilling soil uniformly to 20 cm depth. **Received four additional tillage (20 cm) and five water applications until 26 April. All plots were tilled on 26 April.

Table 3 . Soil nitrate as influenced by soil/crop management during the dry to wet transition in a rice lowland.

Management		Soil nitrate (0-60 cm)			
Dry season	Dry/wet transition	26 Apr*	23 May	13 Jul**	27 Jul***
			- - - - - kg N ha^{-1} - - - - -		
Weedy		14			
	Sesbania		37	20	9
	Mungbean		32	21	10
	Weeds		27	12	8
	Weed free		32	28	9
Weed free		73			
	Sesbania		60	25	8
	Mungbean		66	24	8
	Weeds		57	13	7
	Weed free		59	32	9
Tilled-Wet/dry		108			
	Sesbania		86	23	8
	Mungbean		72	21	9
	Weeds		57	9	9
	Weed free		100	56	10

*Tillage before planting of legumes. **Harvest of legumes. ***One week after soil flooding for rice production.

Table 4 . Nitrogen accumulation by weeds and legumes and N derived through BNF by legumes during the dry to wet transition as influenced by the preceding dry season management in a rice lowland.

Crop in dry/ wet tran- sition	Management during the preceding dry season*	Soil nitrate at planting	Total plant N	N from BNF** Amount	N from BNF** % of total	N uptake from soil
		- - - - kg N ha^{-1} - - - -			%	kg N ha^{-1}
Sesbania	Weedy	14	200	154	77	46
	Weed free	73	213	154	72	59
	Tilled-wet/dry	108	222	125	56	97
Mungbean	Weedy	14	97	63	65	33
	Weed free	73	98	52	54	46
	Tilled-wet/dry	108	114	47	40	66
Weed	Weedy	14	31	0	0	31
	Weed free	73	34	0	0	34
	Tilled-wet/dry	108	35	0	0	35

*Management was initiated on 1 February after tilling soil uniformly to 20 cm depth. Tilled-wet/dry fallow received four additional tillage (20 cm) and five water applications until 26 April. All plots were tilled on 26 April and legumes were planted on 2 May. **Determined by ^{15}N dilution using weeds as reference.

Optimization of Rice Based Crop Systems in Negros Occidental, Philippines: Developing Selection Criteria for Sequentially Cropped Legumes

The benefits from legume cultivation in the crop system are determined by management and environmental impacts on legume growth and BNF. Since legumes are usually a secondary component of the crop system, viable management options for legumes must be low cost. Through a project supported by USAID and implemented by PCARRD, Philippine Tobacco Authority, Tarlac College of Agriculture, UPLB, and NifTAL, a series of experiments was designed to evaluate the impact of rice crop duration on the yield and BNF of sequentially cropped legumes. This project, originally located in Negros, has been relocated to Ilocos Norte and Tarlac due to security problems. Typhoons and volcanic activity have adversely affected the 1989 and 1991 crop seasons.

The project is based on the premise that yield of rain fed crop systems in climates with a distinct dry season is limited by water. Farmers can thus maximize income by allocating more of the limiting water resource to crops offering more favorable prices. By planting primary rice crops of differing durations, the farmer can allocate additional moisture resources to sequentially planted legumes. Depending on relative prices and yields of the component varieties, farmers may maximize income by their selections of rice varieties and the legume species.

In 1990 two rice varieties, IR72 (92-100 days) and IR74 (120-123 days) were planted in Ilocos Norte and Tarlac. Climate data, soil moisture, and pan evaporation were monitored. After harvest of the rice crop, three legume species (inoculated and un-inoculated) were planted in sub-subplots of each rice variety main plot.

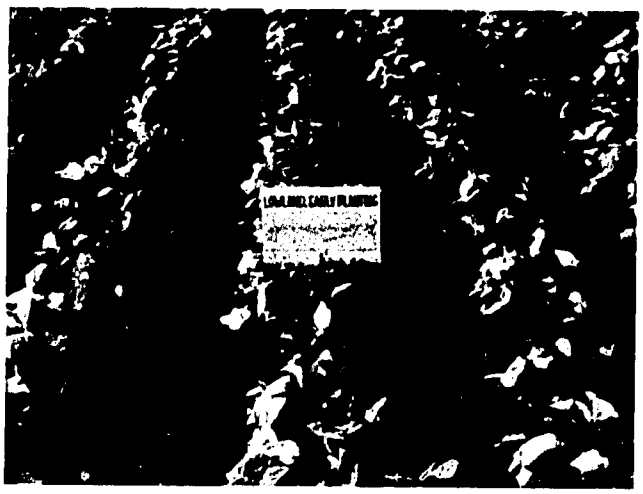
Ilocos is a region of low and sometimes irregular rainfall. The earlier maturing variety, IR72, had a distinct advantage in this climate since water resources were limited during the later stages of maturity for IR74. In contrast, IR74 outperformed IR72 since the longer crop duration could be translated into yield under conditions of greater rainfall (**Table 5**).

Soil moisture contents were higher and legumes tended to yield more when they followed IR72 compared to IR74 since the earlier harvest of IR72 conserved moisture resources for the legume. The benefit of short duration rice varieties was especially evident at Ilocos Norte. IR72 yielded more than the long duration variety, and the conservation of soil moisture generated the greatest response in subsequent legume yields for all but Phaseolus vulgaris. The rainy season at Tarlac is sufficiently long for farmers to benefit from longer duration rice varieties with little benefit for legume culture. Soil moisture and rainfall are excessive and not conducive for good legume yields following rice at that location.

The response to inoculation with rhizobia was significant for soybean at both sites, and for mungbean following IR72 at Ilocos (**Table 6**). Proper selection of primary crop duration in a sequential crop system and inoculation of legume crops are two cost effective methods to maximize yield and income of the entire crop system. These preliminary results indicate the importance of identifying low cost technologies (varietal selection and inoculation) to enhance farm income.

Table 5. Yield of an early maturing (IR72) and late maturing (IR74) rice variety in Ilocos Norte and Tarlac, Philippines.

Rice Variety	Ilocos Norte	Tarlac
	Kg ha^{-1}	
IR 72 -early	5500	3620
IR 74 -late	3310	5500



Soybean planted after lowland paddy rice in Ilocos Norte, Philippines. Soybean on left followed IR 72, an early-maturing IRRI, while the field on the right followed later maturing IR 74. Enhanced growth and BNF are captured through greater water availability following the IR 72 while yield of the paddy is relatively little affected.

Table 6. Seed yield of three legumes grown after rice in Ilocos Norte and Tarlac, Philippines.

Preceding Rice Variety	Legume After Rice	Region: Ilocos Norte		Tarlac		
		Inoc	Uninoc	Inoc	Uninoc	Mean
				kg ha^{-1}		
IR72	Mungbean	1428	1195	745	787	1039
	Soybean	680	530	1319	919	862
	Phaseolus	653	991			822
IR74	Mungbean	606	613	655	552	607
	Soybean	471	398	704	628	550
	Phaseolus	903	914			909

On Farm Trials in Ilocos Norte and Isabella, Philippines, to Evaluate the Benefit of Starter Nitrogen and Rhizobial Inoculation on Mungbean

Prior to the onset of BNF, legumes can experience nitrogen deficiency unless mineral N sources are available. The application of "starter nitrogen" fertilizer is frequently recommended as assurance of N availability during early crop growth. Yet, the economic benefits of such recommendations, compared to inoculation alone, have not been convincingly demonstrated. Soils often have sufficient mineral nitrogen or residual N from previously fertilized non-legume crops. Fertilizer N can be easily leached prior to development of legume roots, and the uptake efficiency of N applied at planting can be very low.

To determine the economic value of applying starter N, series of on-farm trials were conducted in Isabella and Ilocos Norte, Philippines. Support for this work was provided by the Philippine Council for Agriculture and Resources Research and Development (PCARRD), the National Tobacco Administration, Ilagan Experiment Station, the University of Philippines at Los Banos, and NifTAL, through a competitive grant from the USAID PSTC program.

The trials used 19 fields planted to mungbean between lowland rice crops. Plots in farmers' fields received three treatments: 1) control - no inoculation and no fertilizer N; 2) plus inoculation, no fertilizer N; 3) plus inoculation, plus 30 kg N ha^{-1} as NH_4NO_3 applied at planting. The N fertilizer was isotopically enriched for ^{15}N so the fertilizer-use-efficiency (FUE, or proportion of fertilizer applied that was assimilated by the crop) could be determined. Native rhizobial populations were high in both regions, thus large yield increases due to inoculation were not expected.

The results of the trial varied by region. There was no response to starter N at Isabella above the control, but the inoculated treatment had reduced yields. The reason for this reduction is unclear. The inoculant application methodology may have reduced seed vigor. On farms in Ilocos Norte, inoculation increased yields above un-inoculated and the starter N treatment (**Table 7**). The average FUE of starter N at all sites was approximately 25%.

At both sites farmers could have made more money by avoiding the use of starter nitrogen in 1990. Although inoculation did not produce consistent increases in yield, the use of inoculant is many times less expensive than starter nitrogen. These results confirm that inoculant should be recommended as an alternate low cost form of assurance that crops will have adequate N to meet yield potential.

Table 7. Seed yield of mungbean on farms in two provinces of the Philippines under three management treatments

Ilocos Norte			Isabella		
Inoculated	Starter N & Inoc	No Inoc No N	Inoculated	Starter N & Inoc	No Inoc No N
kg ha^{-1}					
981a*	883b	960a	1526b	1634a	1636a

* means within an Province not followed by the same letter differ significantly at $P < 0.05$ by LSD. Means result of 10 and 9 farms in Ilocos Norte and Isabella respectively.

Phosphorus Inputs Affect BNF in the Tropics

Phosphorus (P) is often the most limiting nutrient for legume crops. In the tropics, P deficiency is especially a problem due to the chemical properties of the highly weathered, acid soils which can "fix" P in forms unavailable to the plant.

Long term studies are being conducted at NifTAL to determine the efficiency of P inputs and their residual effects in the high P-fixing Ultisols typical of the tropics. Field experiments are currently in the seventh cropping cycle (three cycles since the last P addition). In each cycle P uptake and crop yields are being monitored. Effective management of the P inputs will mean lower costs of production, and sustainable cropping systems over time.

Another aspect of this research is the study of the effects of nodulation and P availability on yield, N fixation, uptake kinetics, and root growth and distribution on the field crops. Legumes require more available P than other crops due to the additional energy requirement in nodulation and N-fixation. The level of nodulation and nodule growth is dependent on P supply. Since P is an immobile nutrient in the soil, uptake is dependent on root length or an association with mycorrhizal fungi which increase the effective root length. Legumes, *Rhizobia* and mycorrhizae form a tripartite relationship in which the plant supplies carbon in exchange for nitrogen from the *Rhizobia* and phosphorus from the mycorrhizae. Under field conditions the soil is heterogenous with respect to P enrichment, therefore a root or mycorrhizal system which can proliferate and exploit enriched P zones should take up P more efficiently. The present work is designed to develop an understanding of how legume roots and mycorrhizae respond to zones of enriched P.

The long term study measures the effect of four different P rates on the growth, N-fixation, and P uptake of nodulating soybeans. A non-nodulating soybean isoline, grown at each P level, is used as a control to determine the quantity of N fixed. A study was done to examine the root systems of these soybeans to see how root length was effected by nodulation, P fertility and zones enriched with P (**Table 8**). The root length was measured at all four P levels at three depths (25-50 cm not shown). The P concentration in the top 12.5 cm was at least twice that of 12.5 to 25 cm depth. In this experiment the nodulating soybeans reached optimum yield levels at the P_2 rate while the non-nodulating soybeans did not show a response to P because N was limiting. Across all P levels the root length density of the nodulating soybean was about 24% less than that of the non-nodulating in the top 12.5 cm. It would appear that nodulation decreased the ability of the plant to take up P due to a smaller root system. It is also indicated that even under P limiting conditions (P_0 and P_1) the nodulated root system did not proliferate in zones enriched with P (0 to 12.5 cm). Thus, the root systems do not appear to respond to enriched P zones even under P limited conditions.

BNF and P interactions are evident in this field experiment at NifTAL's Kuiaha site.

In further studies to be conducted in pots, we plan to test the hypothesis that the mycorrhizal component of the system is the dynamic component. Despite a smaller root system, the legume, due to the tripartite relationship, is able to support a large mycorrhizal network. The mycorrhizae are able to proliferate in zones of high P and exploit these areas instead of the roots. These studies will be done with several legume species and varieties to examine the variation which exists. An understanding of how legumes meet their P requirement could lead to management practices and species or variety selection which optimize P uptake.

Table 8 . Phosphorous and root distribution for nodulating (NOD) and non-nodulating (NNOD) soybeans at four different P fertilizer rates and two soil depths.

P Level	NOD/ NNOD	Extractable P* 0-12.5cm	Extractable P* 12.5-25cm	Root length 0-12.5cm	Root length 12.5-25cm	% Total roots in 0-12.5cm
		ug P/g soil		cm root/cm³ soil		%
P_0**	NOD	0.88	0.47	2.91	2.79	51.1
	NNOD	0.86	0.57	3.53	3.19	52.5
P_1	NOD	1.93	0.88	3.04	2.66	53.3
	NNNOD	2.09	0.94	3.54	2.79	56.0
P_2	NOD	3.62	1.15	2.45	1.97	55.4
	NNOD	4.10	1.12	3.77	3.01	55.6
P_3	NOD	12.60	3.49	2.57	2.74	48.4
	NNOD	13.06	2.31	3.54	3.27	52.0
				***	**	
$LSD_{0.05}$	***			0.36	0.35	

* Soil P determined by double acid extraction method

** P levels established by incremental addition over six crop cycles of P_0=0, P_1=155, P_2= 310, P_3=930 kg P ha^{-1} (Total P additions)

*** LSD is for the comparison of subplots within the same main plot (P rates)

Predicting Legume Inoculation Requirements in Tropical Cropping Systems

The Soil Science Division of the Department of Agriculture in Thailand, Khon Kaen University, and NifTAL are collaborating on a PSTC project to enhance ecologically based models of legume response to inoculation. Previous studies by NifTAL demonstrated that measurable environmental parameters, primarily soil indigenous rhizobial populations and soil N availability, were the principal determinants of legume response to inoculation. The PSTC project will strive to link these environmental factors to the various cropping systems available in the tropics.

The project's first objective was to determine the status of indigenous rhizobial populations under diverse cropping systems in four eco-regions of Thailand. Researchers found that cropping systems and climatic conditions influenced the populations of native rhizobia both in terms of population density and species diversity. While it is known that cultivation practices and crop systems influence rhizobial populations, this project is the first to quantify the effect of crop systems. Crop systems specific to upland and lowland conditions of S.E. Asia were identified and farms representative of each environment have been selected for study. Three farms for each cropping system within a region will provide replication. Populations of rhizobia will be monitored at identical times during the crop cycle at each farm.

Data will be fit to various soil and climate parameters and variables representing cultural practices such as legume cultivation frequency. The ultimate goal is to develop a more quantitative understanding of when and where inoculation will improve farm yields of legumes. These models will be linked with existing models describing the response to legume inoculation. The model refinement accomplished under this project will enable extension specialists to further advise farmers about the need to inoculate their legume crops.

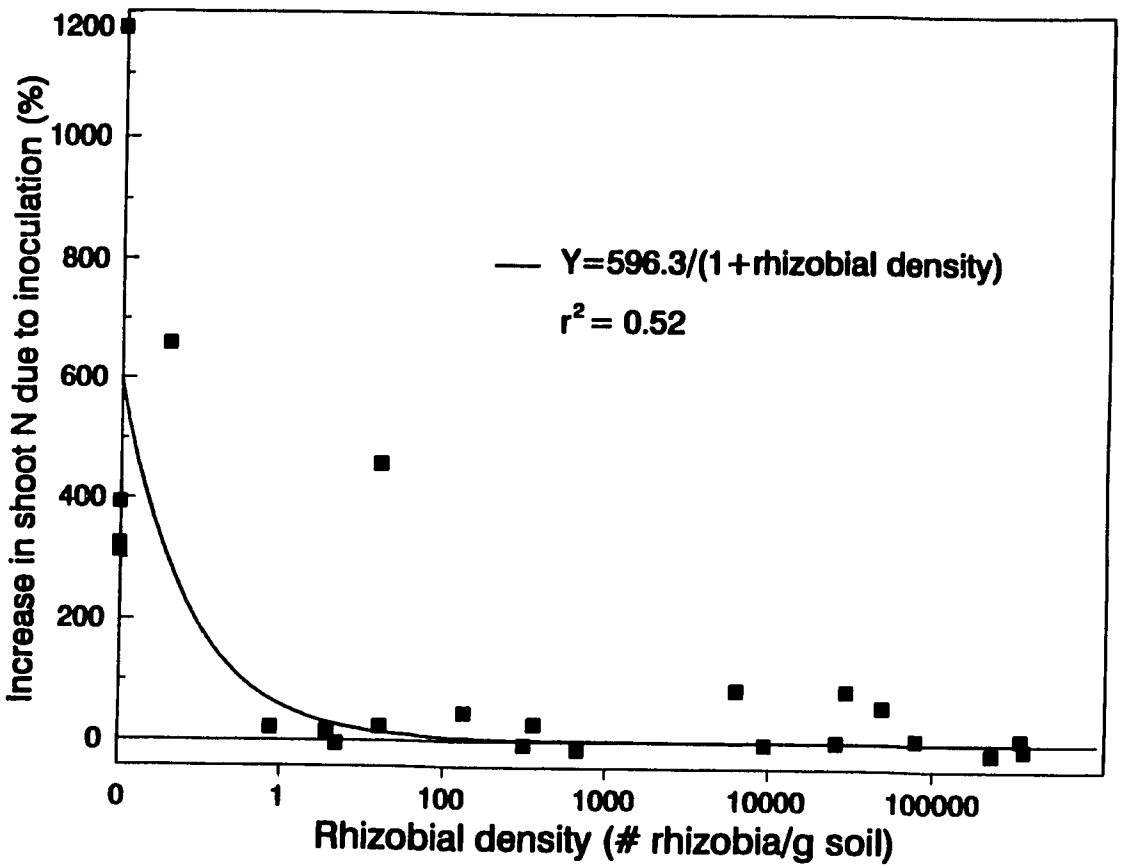
Response of Tree Legumes to Rhizobial Inoculation in Relation to the Population Density of Indigenous Rhizobia

Previous studies at NifTAL resulted in first generation mathematical models to predict the response to inoculation. In these models, the most influential determinant of inoculation response is the population density of indigenous rhizobia that are compatible with the host. Most of these models were based on field trials using grain legumes.

The study has been extended to determine the relationship between yield response to inoculation and rhizobial population density for leguminous trees. Inoculation experiments were conducted in pots containing four soils and tree species *Acacia auriculiformis, A. mangium, A. mearnsii, Leucaena diversifolia, Robinia pseudoacacia,* and *Sesbania grandiflora.*

As in previous models, the magnitude of the response was inversely related to the density of rhizobia in the soil, with the greatest responses occurring where rhizobial densities were less than 50 rhizobia g^{-1} soil. Tree species known to nodulate effectively with *Rhizobium* had a higher proportion of significant responses to inoculation than species known to nodulate effectively with *Bradyrhizobium*. *R. pseudoacacia* and *A. mearnsii* responded significantly to inoculation in several soils despite the presence of more than 1000 rhizobia g^{-1} soil. A hyperbolic model (**Figure 2**) best described the relationship of the increase in shoot dry weight due to rhizobial inoculation to the density of indigenous rhizobia. Incorporation of an index of available soil N into this model improved the fit, indicating the important role of mineral N in mediating responses to rhizobial inoculation. These results indicate that bioassays of soil rhizobial populations can be used to predict responses to inoculation during early seedling growth for certain, but not all, tree species.

Figure 2.


1200
1000
800
600
400
200
0
Increase in shoot N due to inoculation (%)
— Y=596.3/(1+rhizobial density)
r² = 0.52
0
1
100
1000
10000
100000
Rhizobial density (# rhizobia/g soil)

Restoring Degraded Land in Hawaii with an Endemic Nitrogen Fixing Tree

Windswept and desolate, the uninhabited Kaho'olawe Island in the Hawaiian archipelago is the epitome of ecological disaster in the tropics. At one time, a unique dryland forest dominated by an endemic leguminous tree, *Erythrina sandwicensis* (wiliwili), grew on the island. Overuse of forest resources, accidental fires, and uncontrolled grazing by feral goats contributed to the devegetation of the island and left the soil vulnerable to the scouring forces of the Pacific trade winds and tropical storms. Today, thousands of acres of the island's summit are characterized by barren hard clay pans dissected by eroded gorges. Only a few isolated pockets of mature wiliwili trees remain.

U.S. Department of Navy Soil Conservationist Dr. Stephanie Aschmann and NifTAL employee Lionel Amoral water wiliwili seedlings planted in hardpan area of Kaho'olawe.

Supported in part by The Nature Conservancy of Hawaii and the U.S. Department of Navy, NifTAL Project's objectives are to identify soil microbial and nutritional constraints which limit the success of wiliwili in recolonizing eroded sites on Kaho'olawe. These eroded soils typically suffer from low fertility and a paucity of the beneficial soil micro-symbionts which contributed to the success of the original ecosystem. Pot and field experiments have demonstrated the benefit of introducing both appropriate rhizobia and vesicular arbuscular mycorrhizal fungi (VAM), and of raising the phosphorus fertility level of the soil.

Results from these studies will assist land stewards in determining suitable management practices for the reintroduction of wiliwili to Kaho'olawe. Current policies to rehabilitate degraded lands emphasize the introduction of alien, fast-growing, multipurpose nitrogen-fixing trees into agroforestry systems. The researchers hope this case will serve as a model for the reestablishment of native species in degraded lands and the preservation of biological diversity throughout the tropics. In addition to encouraging the regeneration of the original associated floral and faunal community, the use of native species can also strengthen the cultural ties of the indigenous peoples to their land.

NifTAL employee Geoff Haines measures wiliwili growth in a managed watershed area of Kaho'olawe planted with grasses and native species.

Host-Rhizobia Interactions:

The complex relationship between plant and microbe continues to be a productive area for research. Discovering highly effective host-strain combinations for tree legumes was a major accomplishment of this program. These results were immediately used to formulate improved inoculants for important tree species in agroforestry programs. Other research focused on long range goals for improving the symbiosis, striving to unravel the mysteries of how plants regulate the nodulation process.

Rhizobia Associated with Endemic Trees of Hawaii

Due to the islands' isolation in the Pacific and the abundance of ecological niches, a rich and diverse native biota has evolved in the Hawaiian archipelago. Endemic leguminous trees are the major species in three distinct forest ecosystems. These nitrogen fixing species are the *Acacia koa* (koa) in the mesic and rainforest areas, the *Sophora chrysophylla* (mamane) in the high altitude, subalpine zones, and *Erythrina sandwicensis* (wiliwili) in the dryland forest.

Under a grant from The Nature Conservancy of Hawaii, NifTAL staff studied the rhizobial associations of these native legumes. A germplasm collection of effective rhizobial symbionts was developed to serve as a resource for inoculants. These select strains have been used for wiliwili seedling nursery operations to serve revegetation efforts by the U.S. Department of Navy on Kahoolawe Island (see **Restoring Degraded Land in Hawaii with an Endemic Nitrogen Fixing Tree**). Additional studies on the distribution of *Bradyrhizobium* spp. associated with koa were conducted along an elevational transect on Haleakala mountain on Maui. Although the bradyrhizobia are generally considered ubiquitous, they were found only in association with their host. These three native tree legumes are integral components of diverse communities of endemic plants, birds, and insects. Sadly, these communities are diminishing in size and scope due to changes in land use and threats by alien species. An understanding of the contributions of the microbial symbionts to these ecosystems may assist conservators in their preservation and restoration.

Rhizobial Specificity of Fast-Growing Tree Legumes

Rhizobial specificity is defined in terms of nodulation and BNF effectiveness characteristics of a group of rhizobia with a host legume. While these specificity or "cross-inoculation" groups are well defined for many grain and forage legumes, relatively little information has been previously available for leguminous trees. In response to the great interest globally in the use of nitrogen-fixing trees, NIfTAL undertook an ambitious program to bridge this information gap.

A series of cross-inoculation experiments were performed under growth room and greenhouse conditions to delineate rhizobial specificity of a variety of tree legumes of importance to agroforestry and reforestation programs (**Table 9**). *Gliricidia sepium, Calliandra calothyrsus, and Leucaena leucocephala* nodulated effectively with rhizobia isolated from each of the three genera. With a few exceptions, *Sesbania grandiflora* and *Robinia pseudoacacia* nodulated effectively only with rhizobial strains isolated from homologous hosts. Considerable specificity was also found among species that nodulate with *Bradyrhizobium*. For example, Acacia mearnsii nodulated with most strains but fixed N_2 effectively with relatively few, while *Acacia mangium* and *Lysiloma latisiliqua* were specific for both nodulation and effectiveness.

This type of knowledge of the host-rhizobial compatabilities for tree legumes is an important tool to predict the need to inoculate, to select species for most-probable-number plant infection assays, and to formulate rhizobial inoculants suitable for a range of tree species.

Table 9. Summary of specificity and effectiveness of fast- and slow-growing rhizobia genera on tree legumes

Bacteria	Comments
Fast-growing rhizobia (genus *Rhizobium*)	
1. *Leucaena* spp. *Gliricidia sepium* *Calliandra calothyrsus*	Strains effective on each species were generally found to be effective on other species in the group
2. *Sesbania sesban* *Sesbania grandiflora* 3. *Robinia pseudoacacia*	In general, species in groups 2 and 3 nodulate effectively only with strains isolated from their respective genera
Slow-growing rhizobia (genus *Bradyrhizobium*)	
1. *Acacia auriculiformis* *Albizia lebbeck* *Paraserianthes falcataria* *Tephrosia candida*	All species nodulated effectively with a wide range of slow-growing strains
2. *Acacia mangium* 3. *Acacia mearnsii*	Only narrow subsets of slow-growing strains were found to be highly effective on Acacia mangium and Acaciamearnsii
Implications: Rhizobia selected for effectiveness on a particular tree species are unlikely to be highly effective on tree species from other effectiveness groups.	

Mechanisms of Host Regulation of Nodulation Affect Interstrain Competition

Interstrain competition can be a major constraint to the successful introduction of superior N-fixing strains into environments with indigenous rhizobia. Visiting scientist Janez Hacin conducted a series of studies to elucidate the regulatory mechanisms involved in nodulation and competition. These studies contribute to our understanding of effective inoculation techniques, and provide a basis to select or genetically engineer superior symbiotic partners.

Two new procedures were developed to identify the earliest symbiotic structures and the bacterial strains within them. Results indicate that interstrain competition patterns are determined during the earliest stages of the infection process, where the rate of infection and nodule initiation by a given strain plays a crucial role. Subsequent nodule development was shown to be regulated by competition for current photosynthate between early and late initiated nodules, and does not significantly affect the initial competition pattern. **Figure 3** depicts the photosynthate partitioning between early and late developing nodules in soybean as monitored by radioactive labeling in a split root system.

Plant/strain interactions during preinfection involving the exchange of symbiotic signals, induced gene expression, and strain attributes such as motility and chemotaxis, thus appear to be critical determinants of infection rates. Ultimately, the competition pattern in functional nodules reflect these combined factors.

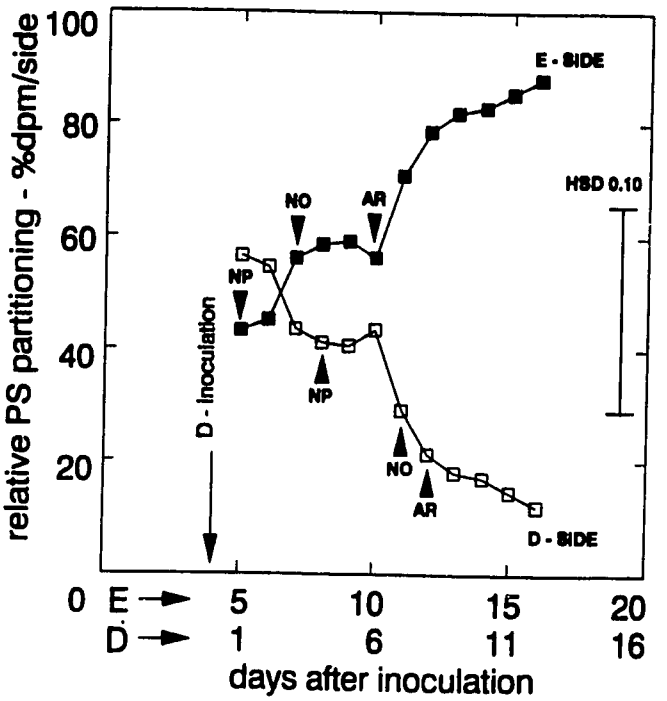


Figure 3. Relative partitioning of labelled photosynthate between early (E) and delayed (D) inoculated root side in a split-root growth system. Arrowheads with symbols indicate the time when individual nodule developmental were first observed; NP=nodule primordia, NO=nodules, AR=acetylene reduction.

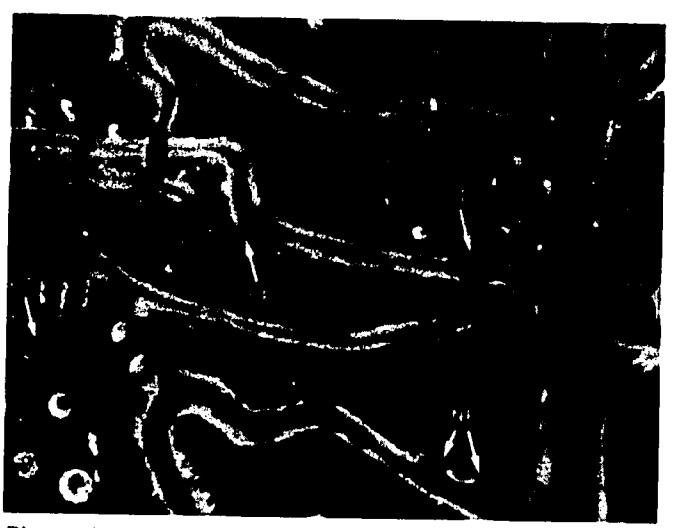


Photo of stained, inoculated root system shows root primordia (RP), root tips (RT), cortical cell divisions (CD), and nodule primordia (NP).

Genetic complementation of *Rhizobium leguminosarum* bv. *phaseoli* mutants defective in nodulation and nodulation suppression.

Legumes invest photosynthate for the formation of root nodules prior to the onset of BNF or the assimilation of fixed N. The host-microbe signals which regulate nodule development are poorly understood. This regulation process has previously been demonstrated using split-root growth systems. Early inoculation of one half-root system by a competent rhizobial strain resulted in the "suppression" of nodule development on the other half-root receiving secondary delayed inoculation. The use of mutants defective in eliciting the suppression response can reveal the stages of nodule development that are critical to regulation, and the genetic components required for the induction of this response.

In this study, five *Rhizobium leguminosarum* bv. *phaseoli* mutants defective at various stages of symbiotic development were used to examine regulation of nodule formation in common beans. Two classes of nodulation defective mutants of *R.l.* bv. *phaseoli* TAL182 that failed to induce nodulation suppression in split root systems were complemented for nodulation ability with wild-type cloned DNA of strain TAL182. A gene library of TAL182 was mass-mated with each of five mutants, and cosmids that corrected the mutations were isolated. The derivatives of three class I mutants that contained cloned DNA from the wild-type strain regained nodulation but not nitrogen fixation abilities, and induced only partial suppression response. When they were used as primary inoculants, there was only 31-68% inhibition of secondary nodulation. In contrast, the derivatives of the two class II mutants regained full nodulation response. Restriction analysis of the overlapping cosmids that partially complemented the class I mutants spanned a 38 kb region and showed a 7.2 kb region in common. The incomplete symbiotic development and limited restoration of nodulation suppression by the complemented class I mutants may be due to a 100 Mdal deletion in one of the four indigenous plasmids. Two complementation groups restored the class I mutants to the nodulation and suppression characteristics of the wild-type strain. A comparison of the suppression phenotypes of the mutants and their derivatives suggests that the extent of the suppression response in common bean may depend on the stage of symbiotic development.

Further studies suggest the suppression response differs in various legumes (**Figure 4**). In alfalfa and siratro, suppression is observed in split-root systems at the early stage of cortical cell division. In soybean, suppression is observed at a later stage. In common bean, suppression is initiated at a stage prior to the onset of nitrogen fixation when rhizobia fully occupy the nodule.

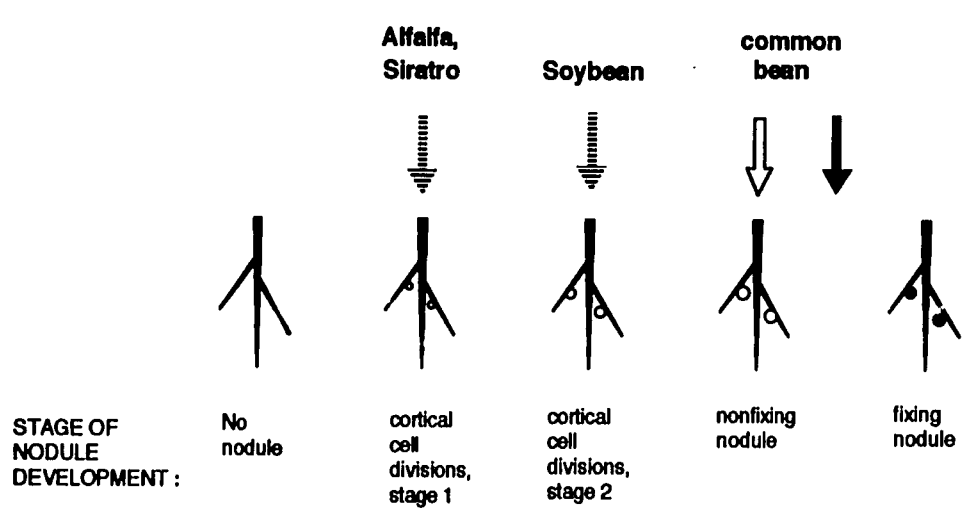


Figure 4. Schematic representation of nodulation phenotypes associated with the suppression responses in different legumes (shaded arrows). The open arrow indicates the onset of suppression in common bean, when rhizobia are inside nodule. The dark arrow indicates full suppression, occuring prior to nitrogen fixation.

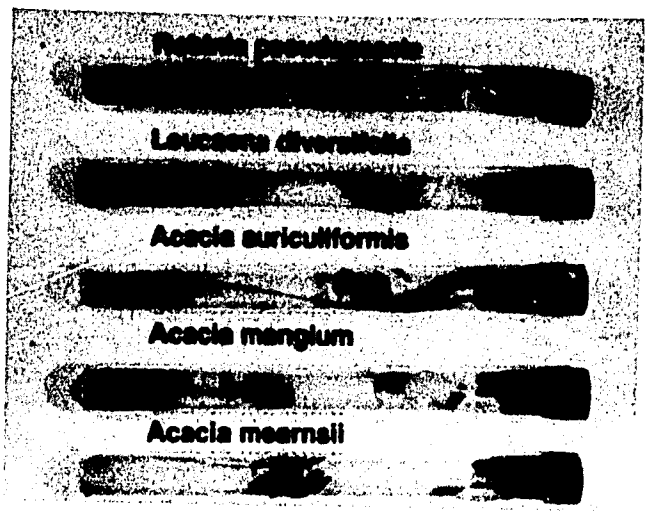
Methodologies of Understanding BNF:

Improvement of BNF is dependent on research that reveals the mechanisms controlling the symbiosis. This research is inturn dependent on the tools to explore this amazing process. We undertook several projects to address methodological constraints to understanding BNF. These included the development of improved methodologies to enumerate tree rhizobia, highly sensitive techniques to monitor genetic changes in rhizobia, and the identification and cloning of essential genetic elements controlling the symbiotic ability of superior rhizobial strains.

Comparison of Most-Probable-Number Estimates of Tree Rhizobia with Plate Counts

Population densities of indigenous soil rhizobia are important determinants of legume response to inoculation. These rhizobia are generally enumerated by most-probable-number (MPN) plant infection assays. There are well established MPN protocols for enumerating soil rhizobia for many economically important grain and pasture legumes, but not for most species of tree legumes.

To ascertain the reliability of results using conventional plant infection growth systems, MPN estimates of rhizobial density were compared with plate counts of pure cultures. Seven tree species were grown in both growth pouches and on agar slants in glass tubes, and seven other species were grown in growth pouches alone. Reasonable agreement was obtained between plate counts and MPN estimates, with closer agreement in agar slants than growth pouches for small-seeded species.



Several species of nitrogen fixing trees nodulate in agar tube growth systems.

Because of slow early root growth in small-seeded trees, only 15 ml of agar was used to make the agar slants. This promoted contact between roots and rhizobia at the bottom of the 25 mm x 250 mm glass tubes. Evaluation of nodulation at weekly intervals suggested that tubes and pouches should be scored for nodulation after at least 5 and 7 weeks respectively. MPN estimates using acacia species in pouches tended to underestimate pure culture plate counts by 100 to over 1000 fold. The possibility of seriously underestimating rhizobial densities with some growth system-tree combinations emphasizes the need to determine the suitability of growth systems prior to enumerating rhizobial populations in soil.

Development of a Highly Sensitive Method that Identifies many *Rhizobium* spp.

Sensitive methods of strain identification are essential to rhizobial ecology and competition studies as well as to inoculant quality control and improvement. Currently, fluorescent antibodies (FA) are used at NifTAL for these purposes; however, FA are limited in their sensitivity in that they are not able to distinguish genetic variation within an identified strain. In addition, cross-reactions of antibodies with other rhizobia sometimes preclude their use in strain identification.

To address the need for sensitive strain identification, we have developed a "DNA fingerprinting" technique based upon the widespread presence of a class of mobile genetic elements called insertion sequences (IS). This technique does not have the constraints and limitations of antibody based methods, and any strain that has one or more copies of the IS element can be identified by this procedure. This method takes advantage of the recently constructed positive selection vectors that entrap IS elements.

The DNA fingerprinting or "IS fingerprint" technique involves several steps: isolation of DNA from an individual strain (purification), cutting purified bacterial DNA with restriction enzymes that recognize particular sequences (digestion), separation of the cut DNA fragments according to their length (fractionation), transfer of the fractionated DNA fragments to a solid support (blotting), and detection of IS elements located on the fractionated DNA pieces of the blot with a labeled copy of the IS (hybridization). The end result, shown in the figure, is a set of patterns similar to grocery store "bar codes". Each bar code pattern is a unique "IS fingerprint" that identifies a rhizobial strain. The IS element that was used as a hybridization probe of this blot was isolated from TAL 1145, a competitive and highly effective inoculant strain which nodulates a wide range of tropical legume trees in the *Leucaena* sp. cross-inoculation group. The distribution of the IS was investigated in *Rhizobium* sp., and found in 14 rhizobial strains used to inoculate the following tropical tree legumes: *Leucaena* sp., *Calliandra* sp., *Pithecellobium* sp., *Prosopis* sp., *Sophora* sp., and *Acacia* sp. The insertion sequence was also found in eight *Rhizobium.* spp. that nodulate either alfalfa or beans and two *Bradyrhizobium* that nodulate soybeans. The isolation of one IS element resulted in the identification and genetic characterization of 24 rhizobial/bradyrhizobial inoculant strains. A sample of some of the characterized strains is shown in **Figure 5**.

Figure 5.

Identification and Cloning of the Symbiotic Region of Superior Rhizobium Strain TAL 1145.

Rhizobium strain TAL1145 is a fast-growing, acid-producing symbiont of *Leucaena leucocephala*. Because of its superior competitiveness and effectiveness, it is an ideal strain for studying the genetics of the rhizobia-tree legume symbiosis. A gene library of TAL1145 was constructed in the cosmid vector pLAFR3, and from this library, nine overlapping cosmid clones (**Figure 6**) were isolated by complementation of nodulation defective mutants of TAL1145 and TAL182 (*Rhizobium leguminosarum* bv. *phaseoli*) and by dot blot hybridization of nodDABCD and nifA genes of *R. meliloti*. A restriction map of the symbiotic region which spanned 38 kb was established. The nodDABC and nifA genes in this region were situated approximately 17 kb apart, with the nodIJ genes between them. These clones enabled a Sym-plasmid cured mutant to form small swellings on the roots of bean plants, whereas they allowed three mutants containing deletions in the Sym plasmid to form ineffective nodules. They also restored the ability of a TAL1145 mutant containing a deletion in the Sym plasmid to nodulate both leucaena and beans. The symbiotic region was located in a 230 Mdal plasmid, the largest of three indigenous plasmids present in this strain. Identification and cloning of the symbiotic region of the strain TAL1145 will help us to transfer these symbiotic genes to other *Rhizobium* strains, and to study their effect on the rhizobia-tree legume symbiosis.

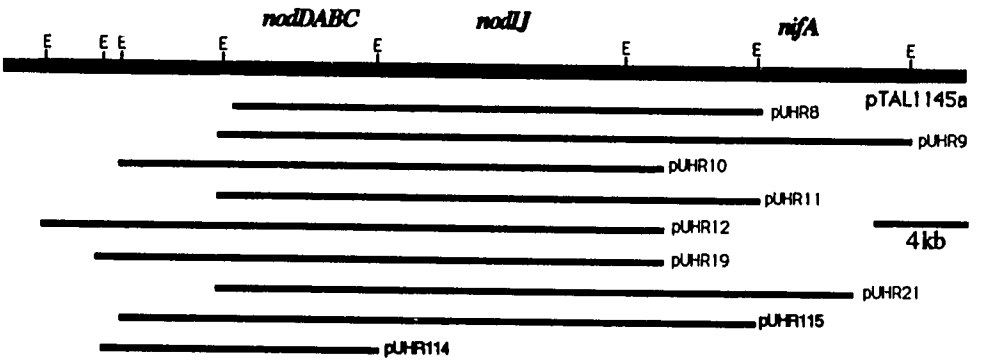


Figure 6. An EcoRI restriction map of the symbiotic region of TAL 1145. The region spanned about 38 kb and hybridized with the nodDABC and nifA genes of *R. meliloti*, and the nodIJ genes of *Rhizobium leguminosarum* bv. *viciae*. The nodDABC and nifA genes in this region were localized approximately 17 kb apart and the nodIJ genes were located in the 11-kb fragment between them. The cosmids pUHR8, pUHR9, pUHR10, pUHR11, and pUHR12 restored nodulation ability to *R. l.* bv. *phaseoli* mutants containing deletions in the Sym plasmid. The cosmids pUHR9 and pUHR10 enabled *R. l.* bv. *phaseoli* 8401, a Sym plasmid-cured strain, to form swellings on the roots of bean plants.

Tools for Development:

The "development" aspects of our R&D program insure NifTAL's relevance to international agriculture. We have created a variety of tools to support the transfer of BNF technology into developing countries. One of our products is a complete training package for extension specialists, including a modular manual, exercises, and resource materials. Many of our tools are outgrowths of our scientific inquiry. The systematic research of WREN was used to develop models to predict the yield response to inoculation. These mathematical models are the basis of an interactive computer program for decision support for commercial and governmental planners. Other computer programs assist prospective inoculant producers with financial analyses for various production scenarios, or with the selection of appropriate rhizobial strains. Our products also include practical, low cost methods to improve the efficiency of inoculant production and research in developing countries.

RESPONSE : Computer Software to Predict the Response to Rhizobial Inoculation

Ecological studies conducted by NifTAL and WREN scientists since 1986 have measured specific environmental parameters which determine the performance of introduced rhizobia. A first generation interactive computer program has evolved from these ecological studies. The programs use mathematical models to predict the magnitude of inoculation response in a given location. The models are based primarily on the population size of indigenous soil rhizobia and the soil N status, factors which have been shown to be the major determinants of inoculation response.

Traditionally, researchers have relied on inoculation trials to measure yield response in the field. Field trials can provide site specific information, but their results are often not transferrable, even to a neighboring field. Compared to field trials, laboratory estimates of rhizobial populations and soil N status are inexpensive and efficient. Large geographical areas can be easily sampled, and with the RESPONSE program, inoculation response can be predicted for each sampling site. In some cases, RESPONSE can estimate inoculation response without direct assays of site soils. Instead, these estimates use other directly observable criteria such as percent legume cover.

When resources for BNF investments are limited, regional planners must target areas where the establishment of inoculant production facilities and extension infrastructure can have the most impact. RESPONSE can assist these decision makers by providing rapid assessments of the potential benefits of legume inoculation in specific environments.

Financial Analyses for Inoculant Manufacturing Enterprises

Realistic projections of financial investments and returns are essential to entrepreneurs who consider establishing inoculant production facilities in the developing tropics. NifTAL has initiated a program to serve commercial companies and government agencies contemplating such an investment. An interactive computer-based program, FAIME (Financial Analyses for Inoculant Manufacturing Enterprises), is being generated to minimize risk and maximize technical and economic efficiency when establishing inoculant production facilities. FAIME provides an analysis of indicators reflecting financial sustainability of the enterprise. The program also provides information to optimize investments in the physical, technical, and labor resources required for various production scales.

FAIME is based on software for small business analyses originally designed by CARE. It has been modified to meet the specialized requirements of the inoculant industry, using information gathered by NifTAL Inoculant Specialist Joseph Rourke and M.B.A. student Marcelo Bartolini. In September, 1991, they conducted a 14 day tour of four Southeast Asian inoculum production facilities. Two of the facilities, Agricultural Laboratories in Sydney, Australia, and Rhizogin-Indonesia, in Jakarta, are private enterprises. The other two are models of government managed facilities. These are the BNF Resource Center in Bangkok, Thailand, administered by the Thailand Department of Agriculture, and facilities at the University of the Philippines, Los Banos.

The evaluation of each site was two-fold: 1) current methods of production, and 2) cost information for start-up, operations, and management. The technical and economic analyses of these various models of production are key to FAIME This self-executing, stand alone program will be another component of NifTAL's package of services to promote BNF agribusiness.

Computer Automated NifTAL Rhizobial Germplasm Catalogue

A computer program is now available to BNF laboratories needing access to NifTAL's extensive rhizobial germplasm catalogue. Information on approximately 2000 strains has been placed in a data base which allows users to request specific outputs. Examples include the legume host, other accession identification numbers, and strain origination. This program is self-executing and requires only an IBM or IBM compatible computer with PC or MS DOS.

This catalogue is part of a series of programs being developed at NifTAL to automate retrieval of information required by routine BNF technology activities. ACCLAIM (ACCounting for Laboratories by Automation and Information Management) is a program which includes data on recommended rhizobial strains for inoculant production, legume seed size/weight relationships and inoculum application rates, legume seed treatment methods, scientific and common names of many legume hosts, and cross-inoculation groups. This software development is part of NifTAL's efforts to provide efficient and current informational resources for BNF researchers.

Joe Rourke and Marcelo Bartolini spent many hours at the computer developing FAIME.

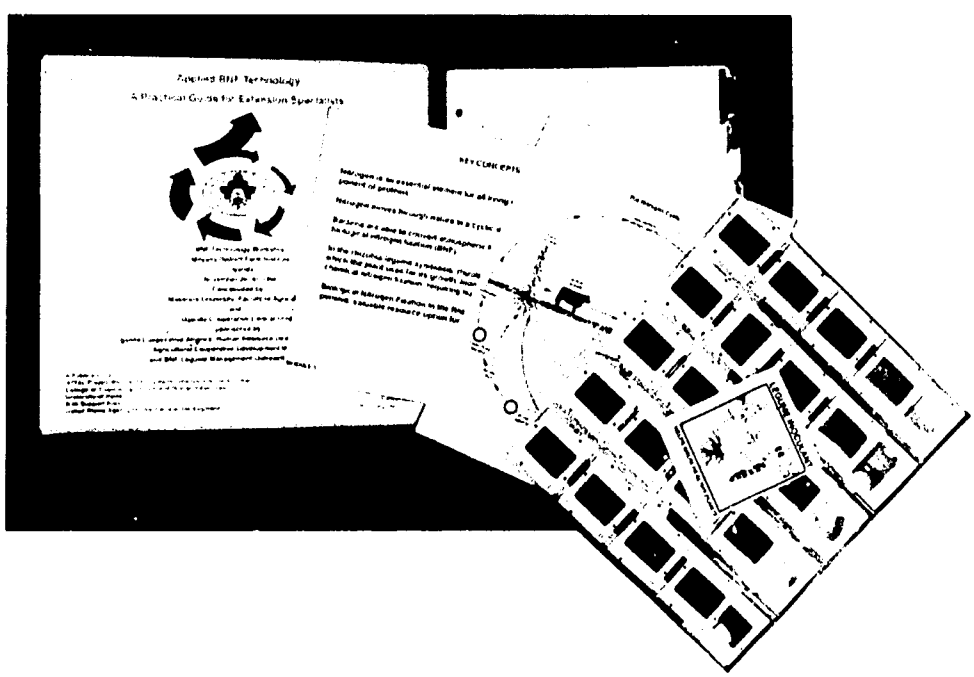
"Applied BNF Technology: A Practical Guide for Extension Specialists"

While critical to developing national BNF programs, quality research alone does not ensure that farmers will benefit from BNF technology. The lack of adequate technology transfer at the extension agent and farmer levels often limits the adoption and exploitation of this appropriate technology. There are several manuals available to train research workers in the techniques and science of legume BNF, but there are few training materials that focus on the extension of BNF technology to farmers.

Communication skills combined with a solid, realistic understanding of the technology are prerequisites for successful technology transfer. NifTAL staff developed a training/resource manual which emphasizes both communication skills and basic information on applied BNF technology. The manual, "Applied BNF Technology: A Practical Guide for Extension Specialists," is structured to support the NifTAL training course for extension specialists.

The manual provides a comprehensive view of the applied aspects of BNF and serves as a valuable reference for extension workers without access to library services. It also features technology transfer materials and methods relevant to extension agents in developing countries. For example, the manual includes simplified concept illustrations and a package of slides that extension agents can use for educational sessions. There are also detailed instructions for field and pot demonstrations to present the potential effects of BNF technology to the farmers.

The manual is organized in modules which highlight key concepts and their relevance to applied BNF technology. A narrative elaborates these key concepts using illustrative materials, and demonstrations augment the narrative. The extension course itself is interactive with hands-on demonstrations and exercises, discussions, self evaluation, and reviews. The manual has been used to train extension specialists from Uganda (1991) and Indonesia (1990), and has been translated into Indonesian. "Applied BNF Technology: A Practical Guide for Extension Specialists" serves as an integral component of NifTAL's technology transfer package.



NifTAL's extension manual and training resources are part of an integrated BNF technology transfer package.

NifTAL Developed Fermentor to Sustain Long-term Testing

NifTAL has designed several models of fermentors for growing rhizobial cells for inoculant production. These fermentors are low cost and low maintenance investments for small to medium-scale inoculant production facilities. NifTAL recommends different models of fermentors as appropriate to the size of the facility and the demand for inoculum. For example, glass fermentors are being used in Haiti, a 20 L fermentor is located in Ethiopia, and 100 L fermentors (**Figure 7**) are used in Thailand, Indonesia, Zambia and Uganda.

In an attempt to integrate minor design alterations and evaluate these changes under actual production conditions, a 100 L NifTAL designed fermentor will be supplied to the Biological Nitrogen Fixation Resource Center (BNFRC) in Bangkok, Thailand. Because the BNFRC produces in excess of 100,000 tons of legume inoculants annually, it is well suited for frequent usage and long-term testing of the fermentor vessel. Dr. Nantakorn Boonkerd, Director, currently employs earlier NifTAL designed fermentors as a part of the BNFRC's regular production.

NifTAL fermentors have been used at NifTAL's prototype inoculant production facilities on Maui and at other BNF facilities worldwide. They have a consistent history of reliability and efficiency as self-contained fermentation systems. Through field testing of improvements, we can ensure the continued quality of the components and the efficiency of the NifTAL fermentation systems.

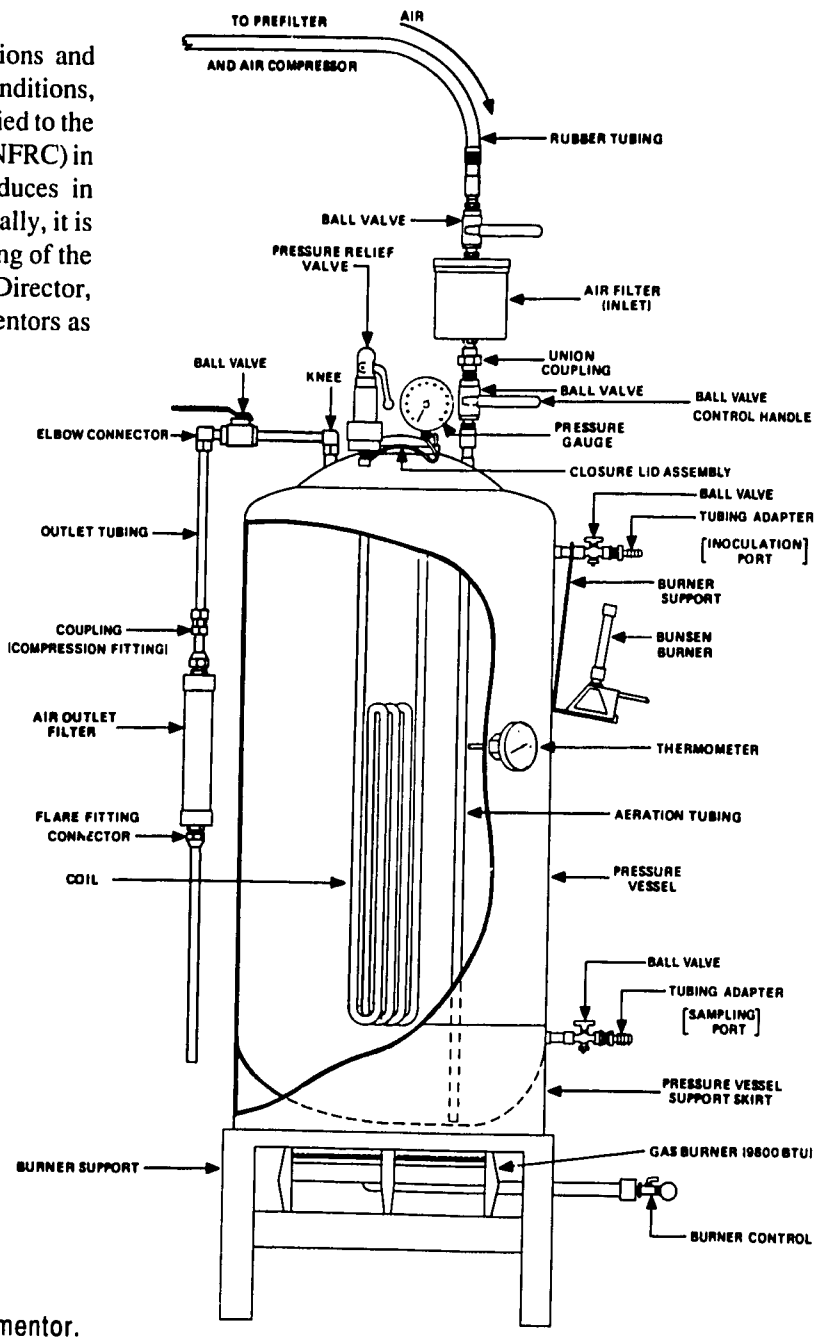


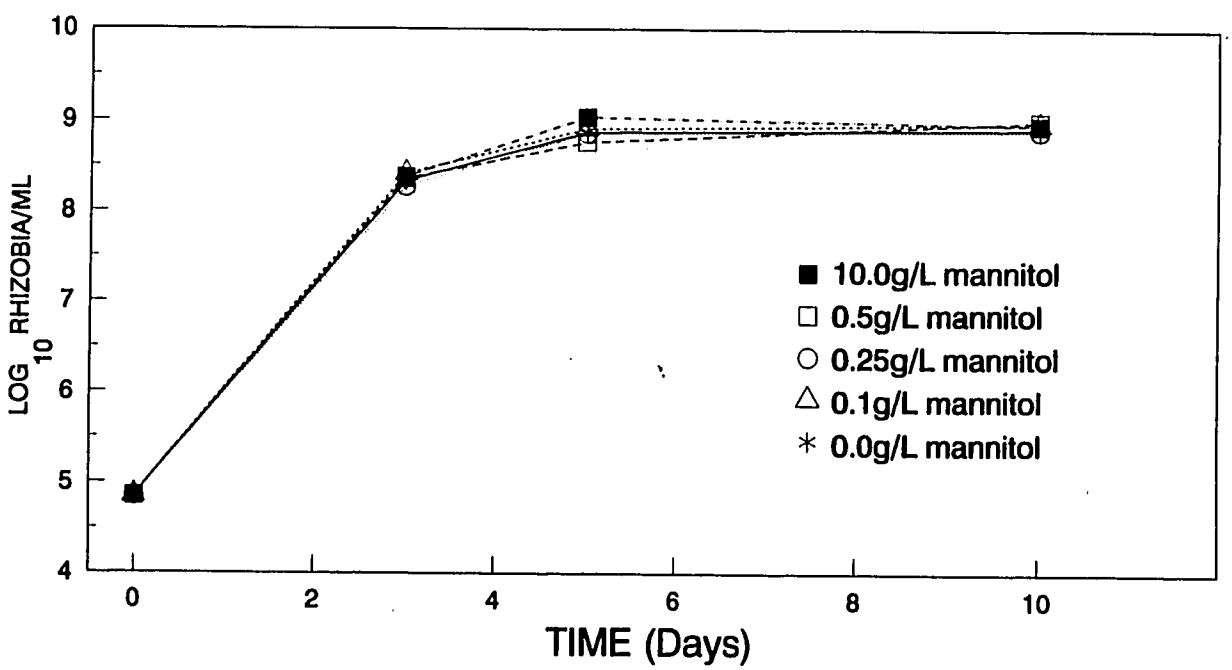
Figure 7. Schematic of NifTAL developed fermentor.

Inoculant Production with Low Levels of Carbohydrates

Growth media for rhizobia routinely contain 10 g per liter of a carbohydrate, 0.5 g per liter yeast extract, and mineral salts. Mannitol is usually recommended as the carbon source, but this material can be expensive to acquire by developing country inoculant producers. To evaluate the feasibility of reducing media costs by inoculant producers, experiments were conducted to examine the efficiency of carbohydrate use by rhizobia.

Bradyrhizobium japonicum TAL 102 (soybean) and *Rhizobium* sp. TAL 1145 (*Leucaena*) were examined for growth response to various carbohydrates in liquid culture and in sterile peat. Tests with mannitol (both strains) (**Figure 8**), glycerol (*B. japonicum*), and sucrose (*Rhizobium* sp.) were conducted in liquid culture. There was virtually no difference in final cell yields with 0, 0.1, 0.25, .5, or 10 g carbohydrate per liter of medium, although growth in peat was generally highest with 10 g per liter. Similar tests with five other species of rhizobia and bradyrhizobia gave the same results. In addition, the bacteria show a direct response to yeast, and do not require carbohydrates for optimum growth when yeast extract is present at 0.5 g per liter. Although growth in peat was generally highest with 10 g per liter of carbohydrate, final cell yields, even with no carbohydrates, were routinely at the high level of 10^9 cells per gram of peat. The organic peat contained sufficient nutrients for optimum growth of the strains, even when cultures were diluted 1000-fold prior to injection into the peat. The initial carbohydrate levels of the culture introduced into the peat did not affect the symbiotic performance of the inoculants when tested on soybean and leucaena. These results indicate that inoculant producers can reduce their media costs without loss of inoculant quality.

Figure 8. Growth of bradyrhizobial strain TAL 102 is not affected by low levels of mannitol in the media after ten days.



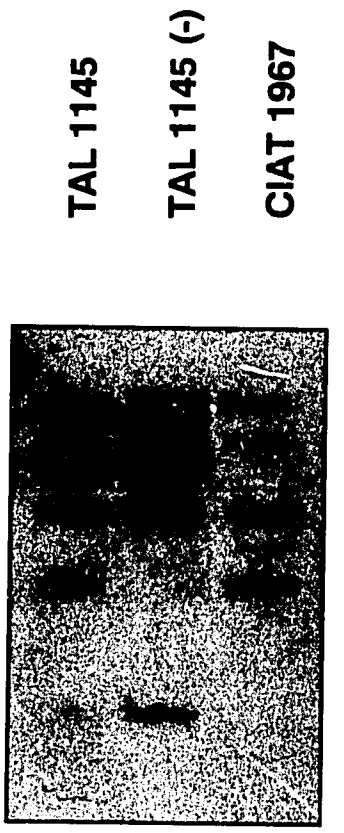
The Use of Rhizobial Insertion Sequence Elements in Quality Control of Inoculants

There is a substantial investment of time and effort to select superior rhizobial strains for use in inoculant formulations. The strains are then generally stored in collections for seasonal use. Among the threats to the integrity of such culture collections are the occurrence of contaminants and genetic changes in the strains during storage, transfer, or growth on non-selective media. Serological methods are used routinely to confirm the identity of specific rhizobial strains. Ideally, inoculant quality control would also include definitive qualitative tests to determine whether the strains retain the essential and desirable traits for which they were selected. These traits include nodulation, nitrogen fixation, competitiveness, host specificity, and persistence. To date, no one method can test all these multifaceted, genetically controlled traits. Some traits are too laborious and time consuming to routinely assay, or are too poorly understood to test.

To explore the feasibility of using the insertion sequence (IS) fingerprinting technique (see **Development of a Highly Sensitive Method that Identifies Many *Rhizobium* spp.** in the **Methodologies for Understanding BNF** section) for inoculant quality control, we tested the ability of IS methods to discern genetic variation in leucaena strain TAL 1145 from different sources. The DNA fingerprints or "IS fingerprints" of current cultures of TAL 1145 from different laboratories were compared to early storage vial cultures and to re-isolates of TAL 1145 that have been in the soil for five and ten years. While the fingerprint pattern was generally very stable among the different sources of the strain, the IS technique clearly identified two variants of TAL 1145 from inoculant storage vials. **Figure 9** shows IS fingerprints of the wildtype strain and those of a non-nodulating mutant and a non-antibody reacting, putative variant of TAL 1145. Missing bands indicates that a stretch of DNA has been deleted. Extra bands indicate that DNA duplication has likely occurred or a mutation has occurred.

This study demonstrates the promise of the IS technique to distinguish genetic variation among cultures of a single strain. Because this method takes only four days to perform, it may have advantages over the plant nodulation tests which require weeks. The study also pointed to the vulnerability of culture collections to genetic change. Rhizobial strains in NifTAL's extensive germplasm collection are stored desiccated or lyophilized in glass ampules. Since no growth occurs, this storage permits long term survival of the bacteria under conditions where genetic change is believed to be minimized.

Figure 9.



Improved MPN Enumeration Systems for Rhizobia.

The ability to quantify populations of rhizobia in non-sterile media and soils is critical to inoculant quality control and field bioassays. There are currently no truly selective media to count rhizobia within the myriad of other micro-organisms found in non-sterile environments. Serological techniques such as immunofluorescent filter counts require expensive equipment, and are not sensitive enough to detect rhizobia at agronomically significant (less than 1000 rhizobia g^{-1} soil) populations levels.

The plant infection most-probable-number technique (MPN) is suitable for both quality control and enumerating rhizobia in soil. The standard MPN technique requires the use of growth pouches for large-seeded grain legumes. Growth assemblies using glass tubes and agar media are suitable for small seeded species.

Agar-tubes are often preferable to growth pouches for MPN's, because of improved counting efficiency and reduction of contamination. Agar tube assemblies are more adaptable to developing country needs. Tubes can be reused and the essential media and supplies are readily available in any laboratory or inoculant production facility. Tubes also have lower space requirements. This space efficiency reduces costs significantly since controlled environment growing areas are expensive to build and operate.

We have begun a project to develop small agar-tube MPN methods for a variety of grain legumes. Following a worldwide search for germplasm, NifTAL has acquired small seeded varieties for *Glycine max* (soybean), *Phaseolus vulgaris* (common bean), *Cicer arietinum* (chickpea), and *Arachis hypogaea* (peanut). Preliminary tests on some accessions indicate they grow and nodulate reliably in small glass agar-tube assemblies. Further studies will be conducted on the germplasm's cross inoculation affinities and on the accuracy of the population estimates.

Our final objective is the design of a simple, inexpensive growth system as well as specific recommendations on the environmental parameters required for its use. Information and germplasm for this new grain legume enumeration system will be distributed to inoculant production facilities and interested research scientists.

Efficient Methods for Biotechnology

The NifTAL biotechnology program is developing, adapting, and testing new, simpler, and less expensive methods that can be used by developing country scientists and others in biotechnology. We have developed two techniques for nucleic acid isolation that do not require an ultracentrifuge, and have tested and routinely use non-radioactive labeling and detection systems for DNA.

Standard techniques used to purify plasmid and genomic DNA usually require the use of an expensive and highly technical ultracentrifuge. This equipment is difficult for developing country scientists to obtain and maintain. We now routinely use rapid plasmid and genomic DNA isolation techniques which do not require an ultracentrifuge, cesium chloride (an expensive chemical), or ethidium bromide (a mutagen). Our DNA isolation techniques require the use of a microcentrifuge, which costs about $1500. This figure represents a significant savings compared to the $80,000 required for an ultracentrifuge and rotors. With our methods, the isolated DNA is produced in less time, and more samples can be handled at once.

Phosphorus-32 (P-32) is a hazardous radioactive isotope that is widely used as a label in molecular biology. Progress has recently been made in finding equally sensitive alternative technologies that do not have P-32's inherent safety and short half-life disadvantages that practically prohibit its use by developing country scientists.

The blot shown in the article on the "**Development of a Highly Sensitive Method that Identifies Many *Rhizobium* spp.**" was performed with our alternative methods. We used digoxigenin labeling of plasmid DNA prepared with a microcentrifuge. Plasmid and bradyrhizobial/rhizobial genomic DNA were prepared with a microcentrifuge. The hybridization probe was digoxigenin labeled and DNA fingerprints were detected through chemiluminescence instead of P-32 technology. All of these alternative techniques save time, money, and do not require sophisticated, expensive equipment.

A Method for Reducing the Cost of Antisera Production

Serological methods for rhizobial strain identification are valuable tools to ensure inoculant quality as well as to monitor the success of the inoculant strains in the field. Both researchers and inoculant producers are often limited in their ability to use these methods because of the high cost of producing the antisera. A major expense involves the maintenance and facilities for the rabbits used in antisera production. These facilities must conform to certain requirements which are costly to maintain.

Traditionally, the antigen of only one rhizobial strain is injected into a rabbit during immunization. To increase the efficiency and reduce costs of antisera production, researchers from NifTAL, the BNFRC, and the Indo-US program evaluated the feasibility of producing antisera by immunizing a single rabbit with three serologically distinct rhizobial strains (**Figure 10**).

Antigens of *Bradyrhizobium japonicum* strain TAL 102 (USDA 110), *Rhizobium* sp. chickpea strain TAL 620, and *Rhizobium* sp. (*Leucaena*) strain TAL 1145 were prepared individually, then mixed to immunize rabbits. Antisera resulting from the mixed antigen immunization were tested against each of the component strains by agglutination, the fluorescent antibody (FA) technique, the enzyme linked immunosorbent assay (ELISA), and the immunoblot technique.

Results from these tests were compared with those obtained with antisera prepared separately against the three individual strains. As shown by the agglutination titres in **Table 10**, the mixed rhizobial species antisera were equal in quality to those produced by conventional methods. Cross absorption of the mixed rhizobial species product yielded strain specific antisera of nearly undiminished quality. The usefulness of the mixed strain antisera was also demonstrated with the other serological techniques. Providing the antigens are serologically distinct, the use of multiple antigens in a single rabbit can lower the costs of facilities, animals, and labor, without sacrificing the effectiveness of the antisera.

Table 10. Agglutination titres of single and mixed rhizobial species antisera

Antigens	Antisera							
	TAL 102[a]	TAL 620[a]	TAL 1145[a]	Mix I[b]	Mix II[d]	TAL 102A[d]	TAL 620A[d]	TAL 1145A[d]
	agglutination titres							
TAL 102[e]	1600	0	0	1600	3200	1600	0	0
TAL 620[f]	0	3200	0	1600	1600	0	800	0
TAL 1145[g]	0	0	1600	1600	3200	0	0	800

[a] Antisera from single rhizobial species immunization at 1×10^4 cells ml^{-1} final antigen concentration
[b] Antisera from mixed rhizobial species (TAL 102 + TAL 620 + TAL 1145) immunization at 1×10^9 ml^{-1} final antigen concentration.
[c] Antisera of the same composition as in "b" but immunization at 3×10^9 cells ml^{-1} final antigen concentration
[d] Same antisera as in "b" but made strain-specific by cross-absorption
[e,f,g] *B. japonicum*, *Rhizobium* sp. (chickpea) and *Rhizobium* sp. (*Leucaena*), respectively

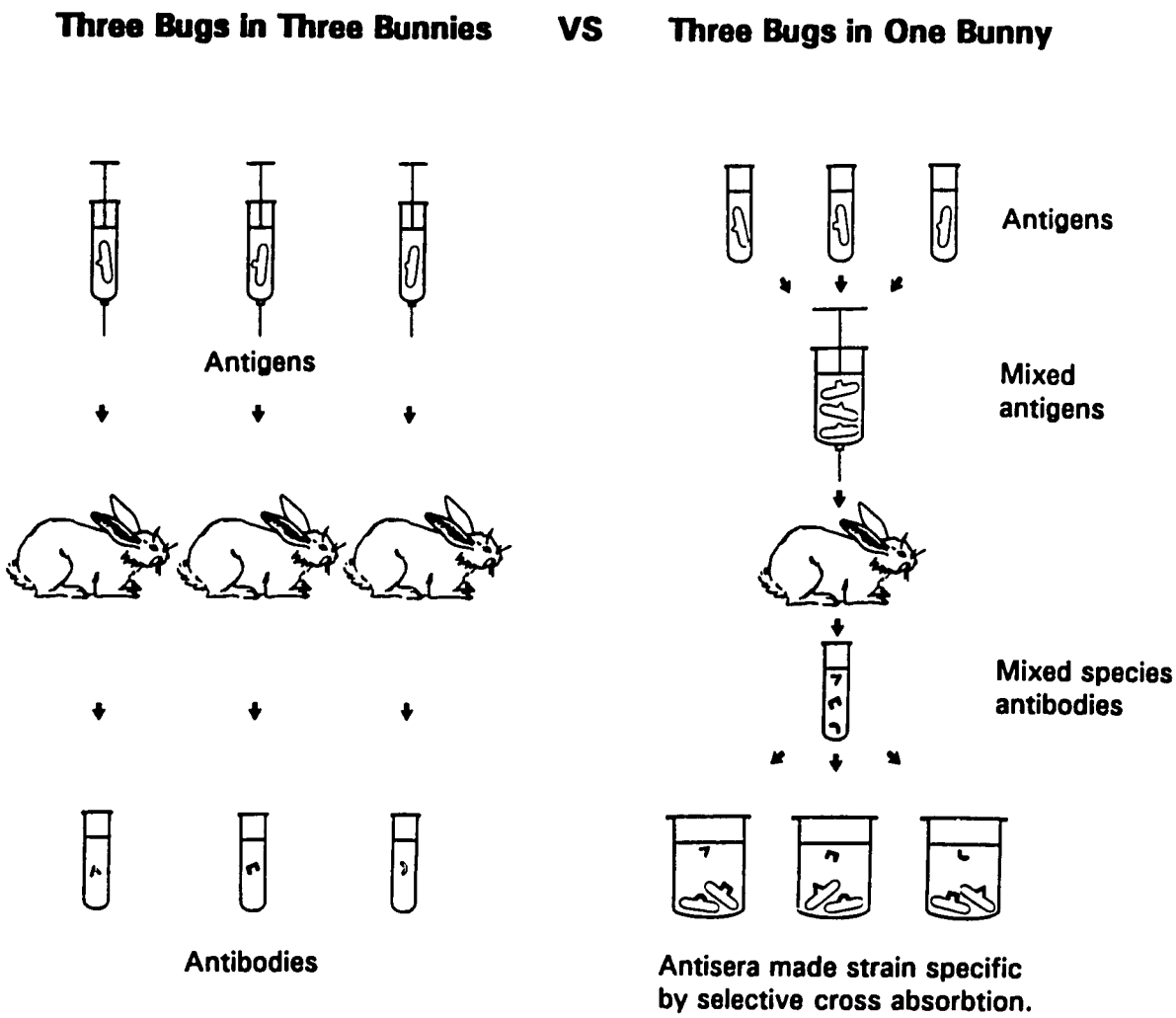


Figure 10. Schematic of traditional and altermate methods of producing antisera.

Appendices

Funds from Outside USAID Core Budget

Contracts and Grants Outside USAID Cooperative Agreement During Reporting Period

Grant Title	Source	Total
Predicting Inoculation Response	NSF/Ecology	750,000
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
Workshop on "BNF Technology Transfer"	USAID	80,000
Development of Acid-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 Evolutionary Adaptation of Symbiotic Nitrogen Fixing Bacteria Associated with Native Hawaiian Leguminous Trees	The Nature Conservancy of Hawaii	8,000
Indo/U.S. STI Workshop	USAID	62,000

University of Hawaii Support of Core Operational Funding

Fiscal Year	85-86	86-87	87-88	88-89	89-90	90-91
Position 1 (Director)	21,250	85,000	85,000	85,000	85,000	85,000
Position 2 (Researcher)		70,000	70,000	70,000	70,000	70,000
Core Operations (Equip/Materials/Supplies)		35,000	50,000	50,000	50,000	50,000
Returned Overhead	29,400	39,300	32,831	49,221	47,502	46,000
Repair and 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	251,000

Abstracts of Published Work (1/1/90-9/30/91)

Abaidoo, R.C., T. George, B.B. Bohlool and P. Singleton. 1990. Influence of elevation and applied nitrogen on rhizosphere colonization and competition for nodule occupancy by different rhizobial strains on field-grown soybean and common bean[1]. Can J Microbiol 36:92-96.

In the absence of indigenous rhizobial populations, the pattern of competition between inoculum strains for nodule occupancy is found to be a stable characteristic, independent of rhizosphere population size, nitrogen application, or elevation. Soybean (*Glycine max* (L.) Merrill) and common bean (*Phaseolus vulgaris* L.), inoculated with peat-based rhizobia, were grown with three nitrogen levels at 320- and 150-m sites along an elevational transect on the island of Maui, Hawaii. Rhizosphere soil of 8-day-old plants was examined by immunofluorescence for populations of three strains each of *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* bv. *phaseoli*, which made up the inocula applied to the respective host legumes at planting. Nodules were examined for occupancy by specific strains at two sampling times. Site differences and nitrogen treatment had no significant effect on rhizosphere colonization or nodule occupancy by the three strains. The three inoculum strains colonized their respective host rhizosphere in approximately equal numbers. In soybean, strain TAL 102 (USDA 110) occupied most of the nodules, while strain TAL 379 (USDA 1366) 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) occupied the least number of nodules. High elevation decreased nodulation by soybean more than that by common bean. Nitrogen application reduced nodule number and mass of both legumes more at the low-elevation than the high-elevation site; the reductions in nodule mass were more pronounced in common bean. The pattern of nodule occupancy remained stable regardless of the differences in nodule number and mass between elevations, N levels, and sampling times.

Caces, M.F., F.M. Robert, D. Borthakur and B.B. Bohlool. 1990. Genetic complementation of *Rhizobium leguminosarum* bv. *phaseoli* Tn5 mutants defective in nodulation and nodulation suppression. Agron Soil Sci .

A plant signal induced by infecting rhizobia is postulated to regulate nodulation and inhibit subsequent nodulation. Evidence for this is seen in a split-root system where a suppression of nodulation occurs on a half root when the other half has been previously nodulated. We investigated the genetics of suppression in *Phaseolus vulgaris* (common bean) using *Rhizobium leguminosarum* bv. *phaseoli* mutants. A highly competitive and effective strain, TAL182, was mutagenized with Tn5 and screened for symbiotic defects. Two classes of nodulation mutants and four types of auxotrophic mutants (Ade$^-$, Leu$^-$, Phe$^-$, Ura$^-$) that are blocked at different stages of symbiotic development, were identified. When the mutants were used as primary inoculants in split-root experiments, they failed to induce nodulation suppression. Complementation of the mutants with a cosmid library of *Rhizobium* sp. TAL1145, restored the wild-type nodulation and suppression characteristics. Restriction analysis of the overlapping clones complementing the Class I and Class II nodulation mutants showed an 8.3-kb region and a 12-kb region in common, respectively.

George, M. and F.M. Robert. 1991. Autoregluatory response of *Phaseolus vulgaris* L. to symbiotic mutants of *Rhizobium leguminosarum* bv. *phaseoli*. Appl and Environ Microbiol, 57(9): 2687-2692.

In *Rhizobium*-legume symbiosis, the plant host controls and optimizes the nodulation process by autoregulation. Tn5 mutants of *Rhizobium leguminosarum* bv. *phaseoli* TAL 182, which are impaired at various stages of symbiotic development, were used to examine autoregulation in the common bean (*Phaseolus vulgaris* L.). Class I mutants were nonnodulating, class II mutants induced small, distinct swellings on the roots, and a class III mutant formed pink, bacterium-containing, but ineffective nodules. A purine mutant (Ade$^-$) was nonnodulating, while a pyrimidine mutant (Ura$^-$) formed small swellings on the roots. Amino acid mutants (Leu$^-$, Phe$^-$, and Cys$^-$) formed mostly empty white nodules. Each of the mutants was used as a primary inoculant on one side of a split-root system to assess its ability to suppress secondary nodulation by the wild type on the other side. All mutants with defects in nodulation ability, regardless of the particular stage of blockage, failed to induce a suppression response from the host. Only the nodulation-competent, bacterium-containing, but ineffective class III mutant induced a suppression response similar to that induced by the wild type. Suppression was correlated with the ability of the microsymbiont to proliferate inside the nodules but not with the ability to initiate nodule formation or the ability to fix nitrogen. Thus, the presence of bacteria inside the nodules may be required for the induction of nodulation suppression in the common bean.

George, T., J.K. Ladha, D.P. Garrity and R.J. Buresh. 1990. Conservation and use of soil and atmospheric nitrogen through legumes in lowland rice-based cropping

systems. Trans 14th Cong Soil Sci, Koyoto, Japan 3:140-145.

Lowland rice culture (rainfed and irrigated) occupies 86% of the total area under rice. Lowland rice growing areas are typically planted to rice during the wet season (WS) and to rice and/or upland crops during the dry season (DS) and transition periods between seasons. Water availability is a main determinant of crops during the DS and the dry-wet transition periods and likewise strongly influences nutrient dynamics, particularly N.

More than 50% of the N used by lowland rice is normally derived from mineralization of soil organic N (Bouldin 1986) and biological nitrogen fixation (BNF) by free-living and rice plant-associated bacteria (Roger and Ladha 1990). The remaining N requirement is provided through fertilizer sources.

Fertilizer N is an expensive input that requires nonrenewable energy resources for its production. Thus, managing of native soil N and BNF for effective use by rice in lowland rice-based cropping systems assumes great significance. This would require understanding the effects of the dry-wet-flooded moisture sequence on retention, gain, and loss of N.

George, T., D.P. Bartholomew and P.W. Singleton. 1990. Effect on temperature and maturity group on phenology of field grown nodulating and nonnodulating soybean isolines. Biotronics 19:49-59.

Lack of sufficient field data on how temperature, photoperiod (latitude), or crop nutrition independently or interactively determines phenological development and yield limit our ability to predict the suitability of soybean (*Glycine max* (L.) Merrill) genotypes for cultivation in varied environments. The effect of temperature and N nutrition on phenology of soybean from four maturity groups was studied at field sites with elevations of 320, 660, and 1050 m on the island of Maui, HI. Four determinate nodulating soybean genotypes representing the maturity groups 00 ('Clay'), IV ('Clark'), VI (D68-0099), and VIII ('Hardee'); one indeterminate genotype of Group VI (N77-4262); and their nonnodulating isolines were planted at each site on the same day. Nonnodulating isolines were used to evaluate the effects of N nutrition on phenology. The mean maximum/minimum air temperatures at the three elevations during the experiment were 27/20, 25/17, and 23/14°C, respectively. Photoperiod decreased from 13 h at planting to 11 h at the last harvest. Soybean maturity was delayed with decreasing temperature mainly due to extended vegetative growth periods averaging 23 and 64% longer at the 660- and 1050-m sites, respectively, compared to the lowest site. Flowering (R1) and physiological maturity (R7) at the highest site were delayed on average by 22 and 25 days, respectively, relative to development at the lowest site. Within each site, duration of the vegetative phase increased with increasing maturity group number presumably due to photoperiodic effects. Higher maturity groups accumulated a greater number of growing degree days (GDD) before R1 at each site than did the lower maturity groups. The GDD requirement before R1 also increased with increasing elevation. The effect of elevation (temperature) on the duration of the seed-fill phase was minimal under similar N nutritional levels. The rate of seed dry matter accumulation (kg ha^{-1} day^{-1}) decreased with increasing elevation, but increased with increasing maturity group number. Nonnodulating isolines flowered later and matured earlier than their nodulating counterparts due to nitrogen insufficiency. These results provide information to refine models predicting phenological development and yield of soybean genotypes for varied environments.

Hoben, H.J., N.N. Aung, P. Somasegaran and U.G. Kang. 1991. Oils as adhesives for seed inoculation and their influence on the survival of *Rhizobium* spp. and *Bradyrhizobium* spp. on inoculated seeds. World J Microbiol Biotech 7:324-330.

Mineral oil, peanut oil and soybean oil were compared with water and gum arabic for their suitability as adhesives for seed inoculation with peat inoculants. Inoculated seeds were stored at 4, 28, and 34°C, and sampled after 1,3 and 9 days to determine the survival of rhizobia. Germination and nodulation tests were performed on the inoculated seeds. Results showed that oils were suitable adhesives for peat inoculants. Although the oils initially bound less inoculant to the seed, the number of surviving rhizobia was similar to that obtained by the gum arabic treatment after storage at 28 and 34°C for 3 and 9 days. An interesting finding of this experiment was that peanut and soybean oils were superior to gum arabic in supporting significantly higher numbers of chickpea rhizobia at 34°C. Inoculated seeds tested for germination and nodulation showed no adverse effects from the oil treatments. Oils hold good potential as adhesives for seed application n inoculation technology.

Kang, U., P. Somasegaran, H.J. Hoben and B.B. Bohlool. 1991. Symbiotic potential, competitiveness, and serological properties of *Bradyrhizobium japonicum* indigenous to Korean soils.. Appl Environ Microbiol 57(4):1038-1045.

The symbiotic potential of *Bradyrhizobium japonicum* isolates indigenous to seven Korean soils was evaluated by inoculating soybeans with 10- and 1,000-fold-diluted soil suspensions (whole-soil inocula). At both levels, signifi-

cant differences in the symbiotic potential of the indigenous *B. japonicum* isolates were demonstrated. The relationship between rhizobial numbers in the whole-soil inocula (x) and nitrogen fixation parameters (y) was best predicted by a straight line ($y=a+bx$) when the numbers in the inocula were 100 to 10,000 ml^{-1}. Thirty isolates from three soils showed wide differences in effectiveness (measured as milligrams of shoot N per plant), and several were of equal or greater effectiveness than reference strain *B. japonicum* isolate YCK 213 and USDA 110 were of equal effectiveness; USDA 110 was the superior strain in colonization (nodule occupancy). Korean isolates YCK 117 and YCK 141 were superior colonizers compared with USDA 110. However, *B. japonicum* USDA 123 was the superior colonizer compared with isolates YCK 213, YCK 141, and YCK 117. In an immunoblot analysis of 97 indigenous Korean isolates of *B. japonicum*, 41% fell into the USDA 110 and USDA 123 serogroups. Serogroups USDA 110 and USDA 123 were represented in six of the seven soils examined. In one Korean soil, 100% of the *B. japonicum* isolates reacted only with antisera of YCK 117, an isolate from the same soil.

Mirza, N.A., B.B. Bohlool and P. Somasegaran. 1990. Non-destructive chlorophyll assay for screening of strains of *Bradyrhizobium japonicum*. Soil Sci Biochem 22(2):203-207.

In greenhouse-grown soybeans [*Glycine max* (L.) Merr. cv Clark] supplied with different amounts of combined nitrogen (0, 75, 300 or 600 mg N as NH_4NO_3), the chlorophyll content of leaf discs was significantly correlated with shoot dry weight (r=0.88; P<0.001). In a parallel experiment with plants inoculated with *Bradyrhizobium japonicum* strains of varying effectiveness, there was a highly significant (P<0.001) correlation between chlorophyll and shoot dry weight (r=0.89); shoot total N (r=0.88): shoot N-fixed (r=0.88); nodule dry weight (r=0.85); and total nitrogenase activity (r=0.79). Effectiveness ranking of strains of *B. japonicum* based on cholorphyll estimates and shoot total N were similar. In field-grown soybean plants, the chlorophyll content of leaf discs was also significantly (r=0.92; P<0.001) correlated with N accumulated in the plant tops. Our data support the use of leaf chlorophyll content as an indirect measure of N status of greenhouse and field-grown soybeans and the effectiveness ranking strains of rhizobia.

Robert, F.M. 1989. Multiple forms of glutamine synthetase in nodules of tropical legumes inoculated with *Bradyrhizobium* and *Rhizobium Fredii* Plant Sci 65:33-38.

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 *Sesbaniaa 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. lunatus* L. but are probably of widespread occurrence in legumes.

Sadowsky, M.J., P.B. Cregan, F. Rodriguez-Quinones and H.H. Keyser. 1990. Microbial influence on gene-for-gene interactions in legume-Rhizobium symbiosis. Plant Soil 129(1):53-60.

Recent advances in our understanding of the molecular genetics of legume-Rhizobium symbioses have indicated that relatively few bacterial genes are required for nodulation. While some of these genes are functionally similar and shared among microsymbionts nodulating genetically diverse legumes, others appear to encode host-specific nodulation (*hsn*) functions which allow for nodulation of plants within a given legume genus. More recently, genotype-specific nodulation (GSN) determinants have been identified in *R. leguminosarum* bv. *viceae* strain TOM and in *B. japonicum* strain USDA 110. GSN determinants refer to those bacterial sequences that allow for nodulation of specific plant genotypes within a given legume species. In contrast to the *avr* loci of several plant pathogens, rhizobial host-range determinants (*hsn* and GSN) have been shown to positively affect nodulation. That is, the introduction of exogenous *hsn* and GSN loci extends host-range. Since GSN loci have been reported to interact with single host plant alleles, it suggests that gene-for-gene interactions occur in rhizobial-legume symbioses and contribute to nodulation specificity at the host genotype level.

Sadowsky, M.J., P.B. Cregan and H.H. Keyser. 1990. DNA hybridization probe for use in determining restricted nodulation among *Bradyrhizobium japonicum* serocluster 123 field isolates. Appl. Enviorn. Microbiol. 56:1768-1774.

Several soybean plant introduction (PI) genotypes have recently been described which restrict nodulation of *Bradyrhizobium japonicum* serocluster 123 in an apparently serogroup-specific manner. While PI 371607 re-

stricts nodulation of strains in serogroup 123 and some in serogroup 127, those in serogroup 129 are not restricted. When DNA regions within and around the *B. japonicum* I-110 common nodulation genes were used as probes to genomic DNA from the serogroup strains USDA 123, USDA 127, and USDA 129, several of the probes differentially hybridized to the nodulation-restricted and -unrestricted strains. One of the gene regions, cloned in plasmid pMJS12, was subsequently shown to hybridize to 4.6-kilobase *Eco*RI fragments from DNAs from nodulation-restricted strains and to larger fragments in nodulation-unrestricted strains. To determine if the different hybridization patterns could be used to predict nodulation restriction, we hybridized pMJS12 to *Eco*RI-digested genomic DNAs from uncharacterized serocluster 123 field isolates. Of the 36 strains examined, 15 were found to have single, major, 4.6-kilobase hybridizing *Eco*RI fragments. When tested for nodulation, 80% (12 of 15) of the strains were correctly predicted to be restricted for nodulation of the PI genotypes. In addition, hybridization patterns obtained with pMJS12 and nodulation phenotypes on PI 371607 indicated that there are at least three types of serogroup 127 strains. Our results suggest that the pMJS12 gene probe may be useful in selecting compatible host-strain combinations and in determining the suitability of field sites for the placement of soybean genotypes containing restrictive nodulation alleles.

Singleton, P.S., B.B. Bohlool and P.L. Nakao. 1990. Legume Response to Rhizobial Inoculation in the Tropics: Myths and Realities. ASA Chapter 8 in Myths and Science of Soils of the Tropics, SSSA Spec. Publ. 29

The myth: tropical legumes, thought to be promiscuous in rhizobial affiliation, do not respond to inoculation. In addition, the presumed ubiquity in the tropics of rhizobia for legumes precludes improving performance of legumes with inoculation. The reality: responses of tropical and temperate legumes to rhizobial inoculation are conditioned by similar ecological factors.

This paper discusses factors affecting the performance of inoculants in the tropics and summarizes results of two global research networks which evaluated inoculation response of different species of legumes. The first, International Network of Legume Inoculation Trials (INLET), demonstrated a positive yield response in 50% of 224 standardized trials with 19 species of legumes in 30 different countries in Africa, Asia and Latin America. A subsequent effort (Worldwide Rhizobial Ecology Network, WREN) attempted to define biotic and abiotic factors conditioning legume response to inoculation. Factors that contributed most to inoculation response were: (1) presence of indigenous rhizobia; (2) nitrogen status of the soil; (3) yield potential of the legume system. A conceptual framework of factors regulating the response to legume inoculation is presented.

Extensive data on the distribution and population densities of rhizobia for five species of legumes in tropical soils are presented and discussed in relation to quantitative relationships between numbers of rhizobia in the soil and response to inoculation. Data presented indicate that many tropical soils have insufficient rhizobia to meet crop nitrogen requirements and therefore require inoculation.

While the ubiquity of the so called "cowpea miscellany" rhizobia in tropical soils has contributed to the myth that tropical legumes do not need inoculation, more recent data reveal strict rhizobial specificity with many important tropical legumes. Bacterial specificity within the tropical legumes and extreme variation in the distribution of tropical rhizobia provide evidence to dispel the myth that tropical legumes do not require inoculation to meet their yield potential.

Somasegaran, P., R.C. Abaidoo and F. Kumaga. 1990. Host-*Bradyrhizobium* relationships and nitrogen-fixation in the Bambarra groundnut [*Voandzeia subterranea* (L.) Thouars nom. cons.]. Trop Agric 67(2):137-142.

23 strains of rhizobia, from 14 leguminous species, were evaluated on a Thai cultivar of *Voandzeia* in Leonard jar trials. The symbiosis ranged from completely ineffective through moderate effectiveness to fully effective. *Bradyrhizobium* sp. TAL 169, isolated from *Vigna unguiculata*, ranked most-effective; the wide-spectrum *Bradyrhizobium* strain CB 756 was ineffective. In a cultivar X *Bradyrhizobium* spp. trial, the host-genotype significantly (P 0.01 or P 0.001) influenced shoot dry weight, shoot N, nodule dry weight and nitrogenase activity. The rhizobial strains were also significant sources of variation for shoot-dry weight (P=0.05), shoot N (p<0.001) and nodule dry weight (P<0.001). There were no significant host X *Rhizobium* interactions, indicating that no specific combination was superior over the broad range of all treatments. Percentage shoot N and specific nitrogenase activity were poor indicators of N_2 fixation capacity in *Voandzeia*. *Voandzeia* cultivar No. 122 P.P. Rust in combination with *Bradyrhizobium* sp. TAL 169 showed high potential for growth, nodulation and N_2 fixation. This study indicated that *Voandzeia* is specific for its rhizobial requirement for effective symbiosis.

Somasegaran, P. and B.B. Bohlool. 1991. The NifTAL Project/MIRCEN and its Contribution to International Cooperation.

NifTAL (Nitrogen fixation by Tropical Agricultural Legumes) is a multi-disciplinary international development support program in tropical agriculture at the University of Hawaii. It is funded mainly by the United States Agency for International Development (USAID). The NifTAL Project was initiated in 1975 and received MIRCEN status in 1982. NifTAL's MIRCEN status is an important commitment to the preservation of NifTAL's comprehensive rhizobial germplasm as an ecological resource. NifTAL's overall goal is to enhance tropical agricultural efficiency through the use of biological nitrogen fixation (BNF) by the legume-rhizobial symbiosis. NifTAL's aim is to enable developing country farmers to reduce their dependence on industrially produced nitrogen fertilizers needed to produce increased quantities of high protein crops for human and animal consumption. To achieve its goal through international cooperation, NifTAL has undertaken a wide range of activities to assist agricultural organizations of the developing countries that have research programs on BNF. These activities include research, networking, technology development, education and training, outreach and information services and rhizobial germplasm and antiserum services. While NifTAL's mandates are focused exclusively on BNF, their implementation is based on a comprehensive agenda that include the following programs: 1) development of genetic technologies for improvement of *Rhizobium*/legume symbiosis for crops and trees; 2) development of methods for monitoring microorganisms in the environment; 3) generation of environmental data bases for predicting symbiotic and saprophytic performance of rhizobia; 4) establishment of regional BNF resource centers; and 5) provisions of technical assistance to commercial inoculant producers. NifTAL's UNESCO-MIRCEN initiative has been especially valuable in computer communication networks, production and publication of communication materials, and supporting participation by nations of countries not eligible for USAID assistance in NifTAL's programs.

Somasegaran, P. and B.B. Bohlool. 1990. Single-strain vs. multistrain inoculation on rhizobial strain effectiveness and competition for nodulation on chickpeas, soybeans, and dry bean. Appl Environ Microbiol 56(11):3298-3303.

The nitrogen-fixing effectiveness of multistrain inoculants was found to be determined by both the effectiveness of the component strains and the percentage of the nodules occupied by them. Multistrain formulations were always either as good as the most effective single-strain inoculant or intermediate between the most and the least effective. The percentage of nodules occupied and the amount of nitrogen fixed by the component strains of a multistrain inoculant showed highly significant linear correlation. The availability of soil N had a significant influence on the nitrogen fixation potential of each strain. The mineral N status of the soil was clearly a significant factor in affecting the competition pattern of *Rhizobium loti* (chick-pea) and *Bradyrhizobium japonicum* strains. Differences between the effectiveness of strains were masked under conditions of soil N availability. However, when soil N was immobilized with sugarcane bagasse, the differences became significant. In the chick-pea system, *R. loti* TAL 1148 (Nit 27A8) was the most effective but not the most competitive of the three strains used. In the soybean and dry bean systems, *B. japonicum* TAL 102 (USDA 110) and *R. leguminosarum* bv. *phaseoli* TAL 182, respectively, were consistently the most effective and, more often than not, the most competitive of the strains used for each species.

Somasegaran, P., R.D. Hirtz and W.J. Broughton. 1990. Rhizobia in tropical legumes: cultural characteristics of *bradyrhizobium* and *Rhizobium* sp. Plant Sci 65:33-38.

We studied the cultural characteristics (carbohydrate utilization and associated pH changes in the media, temperature and pH optima, as well as flagellation patterns) of 51 *Rhizobium* sp. and 27 *Bradyrhizobium* sp. symbionts of tropical legumes. Both fast- and slow-growing rhizobia were isolated from nodules of *Desmodium heterophyllum* and *D. uncinatum*. Contrary to previous data, two-thirds of the bradyrhizobia could use disaccharides as the sole carbon-source. Twice as many *Rhizobium* sp. as *Bradyrhizobium* sp. utilized inositol and trehalose, suggesting that they might have some use in differentiating between the genera. The extent to which bradyrhizobia raised the pH of media was inversely correlated with the initial pH (no change at pH 7). A number of bradyrhizobia could, in combination with various carbohydrates, lower the pH, suggesting that some *Bradyrhizobium* sp. are able to modify their excretion products in response to environmental signals. Some *Rhizobium* sp. possessed only a single, subpolar flagellum. Our data thus demonstrate that *Rhizobium* sp. possessed only a single, subpolar flagellum. Our data thus demonstrate that *Rhizobium* sp. associated with tropical legumes are as heterogeneous as bradyrhizobia of the "cowpea miscellany".

Somasegaran, P., H.J. Hoben and L. Lewinson. 1990. Symbiotic interactions of *Phaseolus acutifolius* and *P. acutifolius* x *P. vulgaris* hybrid progenies in symbiosis with *Bradyrhizobium* spp. and *Rhizobium leguminosarum* bv. *phaseoli*. Can J Microbiol 37(7):497-503

The rhizobial requirements and the N_2-fixing potential of *Phaseolus acutifolius* and *P. acutifolius* x *P. vulgaris* hybrid progenies were investigated in glasshouse experiments. Although *P. acutifolius* was promiscuous, effective

nodulation and N_2 fixation occurred only with a few *Bradyrhizobium* isolates especially those that were isolated from *P. acutifolius, P. limensis, P. penduratus, P. lunatus, Canavalia ensiformis, Calopogonium mucunoides* and *Psophocarpus tetragonolobus*. Strong host x *Bradyrhizobium* interactions were detected in four genotypes of *P. acutifolius* tested against eight isolates of bradyrhizobia and a commercial mixed inoculant. All seven progenies tested nodulated with *Bradyrhizobium* isolats and *Rhizobium leguminosarum* bv. *phaseoli* but there was a highly specific rhizobial requirement for effective nodulation and N_2 fixation. The progeny x rhizobial interaction accounted for 83% of the total phenotypic variation. Two (P-6 and P-7) and five (P-1, P-2, P-3, P-4 and P-5) progenies nodulated and fixed N_2 effectively with *R.I.* bv. *phaseoli* and *Bradyrhizobium* spp., respectively. The *R.I.* bv. *phaseoli*-progeny symbiosis had a greater N_2-fixing potential than the *Bradyrhizobium* isolate-progeny symbiosis. In a soil (oxisol) test, progenies P-6 and P-7 showed significant response to inoculation with *R.I.* bv. *phaseoli*. Strain *R.I.* bv. *phaseoli* TAL 182 was the most competitive strain, occupying 84% of the nodules in both the progenies.

Somasegaran, P. 1991. Inoculant production with emphasis on choice of carriers, methods of production, and reliability testing/quality assurance guidelines. Expert Consultation on Legume Inoculant Production and Quality Control, U.N. FAO, 1991. (In Press)

The value of legumes in improving and sustaining soil fertility was well known to agriculturists but it was the work of Lawes and Gilbert (1891) that showed the property to add nitrogen to the soil was inherent in legumes. Hellriegel and Wilfarth (1888) demonstrated that gains in nitrogen in peas took place only in the presence of soil microorganisms and that the root nodules of legumes were necessary. Finally, Beijernick (1888) isolated the nitrogen-fixing bacteria in the root nodules and the name *Rhizobium* is now given to these organisms. Early methods of introducing rhizobia to benefit legume cultivation involved top-dressing new fields with tons of soil from fields where the legume(s) in question grew well (Fred *et al.*, 1932). This cumbersome soil transfer method was probably in many ways responsible for the birth of the present day inoculant production industry.

The symbiotic association between legumes and rhizobia is by far the most important contributor to the world's supply of biologically fixed nitrogen to agriculture. Effective symbiosis can only be achieved when the nodules are formed by efficient and effective rhizobia. Effectively nodulated legumes can fix substantial amounts of nitrogen (LaRue and Patterson, 1981). The process of biological nitrogen fixation (BNF) and its potential for increasing protein production by legume inoculation has been discussed by Dawson (1970) as one of the means of providing improved nutrition in developing countries.

The first rhizobial inoculants were pure cultures of rhizobia growing on solid medium and these were marketed in 8-10 ounce glass bottles (Fred *et al.* 1932). Since then, the methods of producing inoculants for commercial application has undergone significant evolution to meet the demands of the present day utilization of BNF technology in agriculture.

Inoculant production may be conceptualized as that final process in the research and development (R&D) of BNF technology whereby the promising rhizobia of high symbiotic potential are mass produced and commercially packaged to be used in the farmer's field with the intention of increasing legume-based agricultural production. In the following discussion, the various aspects of BNF technology that relate to the process of inoculant production are discussed.

Thies, J.E., P.W. Singleton and B.B. Bohlool. 1991. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Appl Environ Microbiol 57(1):29-37.

Indigenous rhizobia in soil present a competition barrier to the establishment of inoculant strains, possibly leading to inoculation failure. In this study, we used the natural diversity of rhizobial species and numbers in our fields to define, in quantitative terms, the relationship between indigenous rhizobial populations and inoculation response. Eight standardized inoculation trials were conducted at five well-characterized field sites on the island of Maui, Hawaii. Soil rhizobial populations ranged from 0 to over 3.5×10^4 g of soil^{-1} for the different legumes used. At each site, no less than four but as many as seven legume species were planted from among the following: soybean (*Glycine max*), lima bean (*Phaseolus lunatus*), cowpea (*Vigna unguiculata*), bush bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*), *leucaena leucocephala*, tinga pea (*Lathyrus tingeatus*), alfalfa (*Medicago sativa*), and clover (*Trifolium repens*). Each legume was (i) inoculated with an equal mixture of three effective strains of homologous rhizobia, (ii) fertilized at high rates with urea, or (iii) left uninoculated. For soybeans, a nonnodulating isoline was used in all trials as the rhizobia-negative control. Inoculation increased economic yield for 22 of the 29 (76%) legume species-site combinations. While the yield increase was greater than 100 kg ha^{-1} in all cases, in only 11 (38%) of the species-site combinations was the increase statistically significant (P 0.05). On average, inoculation increased yield by 62%.

Soybean (*G. max*) responded to inoculation most frequently, while cowpea (*V. unguiculata*) failed to respond in all trials. Inoculation responses in the other legumes were site dependent. The response to inoculation and the competitive success of inoculant rhizobia were inversely related to numbers of indigenous rhizobia. As few as 50 rhizobia g of soil $^{-1}$ eliminated inoculation response. When fewer than 10 indigenous rhizobia g of soil $^{-1}$ were present, economic yield was significantly increased 85% of the time. Yield was significantly increased in only 6% of the observations when numbers of indigenous rhizobia were greater than 10 cells g of soil^{-1}. A significant response to N application, significant increases in nodule parameters, and greater than 50% nodule occupancy by inoculant rhizobia did not necessarily coincide with significant inoculation responses. No less than a doubling of nodule mass and 66% nodule occupancy by inoculant rhizobia were required to significantly increase the yield of inoculated crops over that of uninoculated crops. However, lack of an inoculation response was common even when inoculum strains occupied the majority of nodules. In these trials, the symbiotic yield of crops was, on average, only 88% of the maximum yield potential, as defined by the fertilizer N treatment. The difference between the yield of N-fertilized crops and that of N_2 fixation capacity of rhizobial strains, and the efficiency of symbiosis. In this study, we show that the probability of enhancing yield with existing inoculation technology decreases dramatically with increasing numbers of indigenous rhizobia.

Thies, J.E., P.W. Singleton and B.B. Bohlool. 1991. Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil Appl Environ Microbiol 57(1):29037.

The ability to predict the symbiotic performance of rhizobia introduced into different environments would allow for a more judicious use of rhizobial inoculants. Data from eight standardized field inoculation trials were used to develop models that could be used to predict the success of rhizobial inoculation in diverse environments based on indices of the size of indigenous rhizobial populations and the availability of mineral N. Inoculation trials were conducted at five diverse sites on the island of Maui, Hawaii, with two to four legumes from among nine species, yielding 29 legume-site observations. The sizes of indigenous rhizobial populations were determined at planting. Soil N mineralization potential, total soil N, N accumulation and seed yield of nonnodulating soybean, and N derived from N_2 fixation in inoculated soybean served as indices of available soil N. Uninoculated, inoculated, and fertilizer N treatments evaluated the impact of indigenous rhizobial populations and soil N availability on inoculation response and crop yield potential. The ability of several mathematical models to describe the inverse relationship between numbers of indigenous rhizobia and legume inoculation responses was evaluated. Power, expoential, and hyperbolic functions yielded similar results; however, the hyperbolic equation provided the best fit of observed to estimated inoculation responses ($r^2 = 0.59$). The fact that 59% of the observed variation in inoculation responses could be accounted for by the relationship of inoculation responses to numbers of indigenous rhizobia illustrates the profound influence that the size of soil rhizobial populations has on the successful use of rhizobial inoculants. In the absence of indigenous rhizobia, the inoculation response was directly proportional to the availability of mineral N. Therefore, the hyperbolic response function was subsequently combined with several indices of soil N availability to generate models for predicting legume inoculation response. Among the models developed, those using either soil N mineralization potential of N derived from N_2 fixation in soybean to express the availability of mineral N were most useful in predicting the success of legume inoculation. Correlation coefficients between observed and estimated inoculation responses were $r = 0.83$ for the model incorporating soil N mineralization potential and $r = 0.96$ for the model incorporating N derived from N_2 fixation. Several equations collectively termed "soil N deficit factors" were also found to be useful in estimating inoculation responses. In general, models using postharvest indices of soil N were better estimators of observed inoculation responses than were those using laboratory measures of soil N availability. However, the latter, while providing less precise estimates, are more versatile because all input variables can be obtained through soil analysis prior to planting. These models should provide researchers, as well as regional planners, with a more precise predictive capability to determine the inoculation requirements of legumes grown in diverse environments.

Thies, J.E., P.W. Singleton and B.B. Bohlool. 1991. Subgroups of the cowpea miscellany - symbiotic specificity within *Bradyrhizobium* spp. for *Vigna unguiculata, Phaseolus lunatus, Arachis hypogaea,* and *Macroptilium atropurpureum.* Appl Environ Microbiol 57(5):1540-1545.

Rhizobia classified as *Bradyrhizobium* spp. comprise a highly heterogeneous group of bacteria that exhibit differential symbiotic characteristics on hosts in the cowpea miscellany cross-inoculation group. To delineate the degree of specificity exhibited by four legumes in the cowpea miscellany, we tested the symbiotic characteristics of indigenous cowpea bradyrhizobia on cowpea (*Vigna unguiculata*), siratro (*Macroptilium atropurpureum*), lima bean (*Phaseolus lunatus*), and peanut (*Arachis hypogaea*). The most-

probable-number counts of indigenous bradyrhizobia at three sites on Maui, Hawaii, were substantially different on the four hosts: highest on siratro, intermediate on cowpea, and significantly lower on both lima bean and peanut. Bradyrhizobia from single cowpea nodules from the most-probable-number assays were inoculated onto the four hosts. Effectiveness patterns of these rhizobia on cowpea followed a normal distribution but were strikingly different on the other legumes. The effectiveness profiles on siratro and cowpea were similar but not identical. The indigenous cowpea-derived bradyrhizobia were of only moderate effectiveness on siratro and were in all cases lower than the inoculant-quality reference strain. Between 5 and 51% of the bradyrhizobia, depending on site, failed to nodulate peanut, whereas 0 to 32% failed to nodulate lima bean. No significant correlation was observed between the relative effectiveness of the bradyrhizobia on cowpea and their corresponding effectiveness on either lima bean or peanut. At all sites, bradyrhizobia that were ineffective on cowpea but that effectively nodulated lima bean, peanut, or both were found. Eighteen percent or fewer of the bradyrhizobia were as effective on lima beans as the reference inoculant strain; 44% or fewer were as effective on peanut as the reference strain. Only 18% of all cowpea-derived bradyrhizobia tested were able to form N_2-fixing nodules on both lima bean and peanut. These results indicate the need to measure indigenous bradyrhizobial population characteristics directly with the crop of interest to obtain an accurate assessment of the need to inoculate.

Thies, J.E., P.W. Singleton and B.B. Bohlool. 1990. Models for predicting legume response to rhizobial inoculation: importance of indigenous rhizobia and soil N. ASA (In Press)

Determining the need to inoculate is an important consideration in the cultivation of leguminous crops. Increasing crop yield through rhizobial inoculation depends on many environmental factors. Eight inoculation trials, using 2-4 legumes in each, were conducted at five diverse sites on the island of Maui, Hawaii, to identify and quantify the factors that determine, and could be used to predict, legume inoculation response. Uninoculated, inoculated, and fertilizer N treatments were used to evaluate the effects of indigenous rhizobia and available soil N, inoculation response, and crop yield potential, respectively. Results allowed development of regression models that can be used to estimate the potential benefit derived from rhizobial inoculation at various locations. Models combine an inverse relationship between numbers of indigenous rhizobia and inoculation response with several measures of soil N to generate inoculation response estimates.

Thies, J.E., B.B. Bohlool and P.W. Singleton. 1991. Environmental effects on rhizobial interstrain competition for nodule occupancy. From the 13th North American Symbiotic Nitrogen Fixation Conference 1991.

Effective use of rhizobial inoculant depends on the ability of inoculant strains to survive in the environment and compete successfully with indigenous rhizobia for nodule occupancy. In eight inoculation trials conducted at five diverse sites on the island of Maui, HI, equal numbers of three serologically distinct strains of effective homologous rhizobia in a peat-based inoculant were applied to seeds of soybean, bush bean, cowpea, lima bean, peanut, leucaena, clover, and tinga pea. We studied the influence of environmental variables on interstrain competition from two perspectives: between the introduced rhizobia as a group and the indigenous population; and among the three introduced strains comprising the inoculum. While temperature, rainfall, and soil fertility were significantly correlated with nodule occupancy by inoculant strains, the most significant environmental variable controlling their competitive success was the size of the indigenous rhizobial population. Ability of several competition models to predict field results was subsequently evaluated and outcomes compared. Nodule occupancy by inoculant rhizobia was best described (r^2=0.51, $p<0.001$) using the model: $\mathbf{y = a + b(\log_{10} x + 1)}$, where $\mathbf{x}$ = number of indigenous rhizobia g^{-1} soil and $\mathbf{y}$ = percent nodule occupancy by inoculant rhizobia. For each legume grown in the trials, one of the three inoculant strains was shown to be a poor competitor at all sites. Competition among the other two strains varied across sites and in several cases could be related to specific environmental variables. The results, however, indicated that competitive strains could be selected that perform well across a range of environments.

Woomer, P., W. Asano and D. Pradha. 1990. Environmental factors related to rhizobial abundance in kikuyugrass (*Pennisetum clandestinum*) pastures. Trop Agric 67(3):217-220.

Population dynamics of legumes and their associated rhizobia were determined in kikuyugrass (*Pennisetum clandestinum* Hochst. ex Choiv.) pastures at four sites on the island of Maui, Hawaii, USA. Ranges in factors measured at the sites which represented three soil great-groups were mean annual rainfall (MAR) 810-1800 mm yr^{-1} pH 5.0-6.5 and total extractable bases 3/7-54 mEq $100g^{-1}$ soil. Competition from kikuyugrass, as estimated by mat thickness of the stolons, significantly reduced the abundance of white clover (*Trifolium repens* L.) but not that of *Desmodium intortum* cv. Greenleaf (Mill.) Fawc.and Rendle. Soil populations of *Rhizobium leguminosarum* bv. *trifolii* and *Bradyrhizobium* sp. ranged 1.78-5/49 (log_{10} g^{-1} soil) and were significantly correlated with increased host legume presence but not with soil pH, total extractable bases (mEq $100g^{-1}$ soil) or MAR.

Woomer, P., J. Bennett and R. Yost. 1990. Overcoming the inflexibility of the most-probable-number procedures. Agron J 82:349-353.

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 presented in this paper are from a computer program, the Most Probable Number Enumeration System (MPNES), which generates solutions and confidence limits (P=0.05) for population estimates of MPN data. 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. The program was also used to generate population estimates from fractional base dilution ratios. The MPNES increases 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 adjusts for inoculation volume and initial dilution ratios. In this way, MPN experiments can be designed to better measure the organism of interest rather than have the design dictated by published tables.

Woomer, P., J.E. Thies and J.W. Rourke. 1990. Models to predict the persistence and symbiotic performance of rhizobia introduced into tropical soils. ASA (In Press)

For legume inoculation to result in enhanced crop yield, introduced rhizobia must be able to persist in the soil environment in sufficient number to infect host plants. Survival studies and inoculation trials conducted at ecologically diverse sites on the island of Maui, HI, identified the primary factors controlling rhizobial persistence and legume response to inoculation. Regression models arising from these studies were used to develop the computer programs PERSIST and RESPONSE. PERSIST is a program designed to predict the survival of introduced rhizobia in tropical soils based on soil taxonomy and other measurable environmental parameters using the Mitscherlich equation. RESPONSE is a companion program designed to provide an estimate of the potential benefit, in terms of increased yield and return on investment, that may be derived from rhizobial inoculation. Estimates of indigenous rhizobial population size and available soil N driv^ equations to predict inoculation response.

Conference Presentations

The National Conference on Plant and Animal Biotechnology. February 25-March 3, 1990, Nairobi, Kenya.

Bohlool B. 1990. Biological nitrogen fixation technology: Potential for Increased Agricultural Production with Lower Inputs.

The 90th Annual Meeting of the American Society for Microbiology. May 13-17, 1990, Anaheim, California.

Caces M., F. Robert and B. Bohlool. 1990. Host nodulation suppression response as a factor in competition among *Rhizobium leguminosarum* bv. *phaseoli* strains.

8th International Congress of Nitrogen Fixation. May 20-26, 1990, Tennessee.

Bohlool B. 1990. Introduction to nitrogen fixation in agriculture and industry: Contribution of BNF to sustainability of agriculture.

Caces M., F. Robert, D. Borthakur and B. Bohlool. 1990. Genetic complementation of *Rhizobium leguminosarum* bv. *phaseoli* Tn5 mutants defective in nodulation and nodulation suppression.

Hacin J., B. Bohlool and P. Singleton. 1990. Photosynthate partitioning and regulation of soybean (*Glycine max* L. Merr.) nodule development.

14th International Congress of Soil Science. August 12-18, 1990, Kyoto, Japan.

George T., J. Ladha, D. Garrity and R. Buresh. 1990. Conservation and use of soil and atmospheric nitrogen through legumes in lowland rice-based cropping systems.

Fourth African Association of Biological Nitrogen Fixation (AABNF). September 25-29, 1990, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Singleton P., B. Bohlool and J. Thies. 1990. Predicting the response to legume inoculation in the tropics.

The International Conference on Agriculture for the 21st Century. October 12, 13, 14, 1990, Wailuku, Maui, Hawaii.

Bohlool B. Ben. 1990. Biological nitrogen fixation: old technology, new appeal.

American Society of Agronomy, Crop Science Society of America and Soil Science Society of America. October 21-26, 1990, San Antonio, TX.

Cassman K., P. Singleton and T. George. 1990. Soybean yield and N_2 fixation in relation to nitrogen, phosphorus and carbon budgets in a tropical soil.

George T. and B. Bohlool. 1990. Relationship between rhizobial numbers and distribution in soil and proportion and total of plant N derived from N_2 fixation by soybean.

Keyser H., D. Turk and P. Somasegaran. 1990. Specificity in the tree legume-rhizobia symbiosis.

Thies J., P. Singleton and B. Bohlool. 1990. Models for predicting legume response to rhizobial inoculation: importance of indigenous rhizobia and soil N.

Woomer P., J. Thies and J. Rourke. 1990. Persist/response: models to predict the persistence and symbiotic performance of rhizobia introduced into tropical soils.

91st General Meeting of the American Society for Microbiology. May 5-9, 1991, Dallas, Texas.

Bohlool B. 1991. Committee on international activities in microbiology, PSAB; AAM - Round Table - Convener

Twelfth Annual Plant Physiology in Hawaii Symposium. May 10, 1991, Honolulu, HI.

Hacin J., B. Bohlool and P. Singleton. 1991. Regulation of nodulation in soybean.

13th North American Symbiotic Nitrogen Fixation Conference. August 25-30, 1991, Banff, Canada.

Abaidoo R., C. Van Kessel and W.G. Akanwariwiak. 1991. Utilization of nitrogen resources in legume-cereal associations.

Boonkerd N., P. Wadisirisuk and V. Thananusont. 1991. Effectiveness variation of indigenous rhizobia under different cropping system in Thailand.

George M., D. Borthakur. 1991. Cloning of the symbiotic region of *Rhizobium* sp. TAL 1145 that nodulates *Leucaena leucocephala* and *Phaseolus vulgaris*.

George T., J.K. Ladha, R.J. Buresh and D.P. Garrity. 1991. The role of legumes in conserving and recycling soil and atmospheric nitrogen in lowland rice-based cropping systems.

Hoben H., P. Somasegaran, N. Boonkerd, Y.D. Gaur and B.B. Bohlool. 1991. Antisera production by immunization with mixed rhizobial species antigen.

Keyser H. and D. Turk. 1991. Rhizobia that nodulate tree legumes.

Rice D., P. Somasegaran, K. MacGlashan and B. Bohlool. 1991. Isolation of a mobile genetic element from a *Leucaena leucocephala rhizobium* and its distribution in other *Rhizobium* spp.

Singleton P., T. George and J. Rourke. 1991. The Relationship between root morphology, rate of NO_3 assimilation, and N_2 fixation by *Glycine max* and *Phaseolus vulgaris*.

Thies J., B. Bohlool and P. Singleton. 1991. Environmental effects of rhizobial interstrain competition for nodule occupancy.

Turk D., H. Keyser and P. Singleton. 1991. Response of tree legumes to rhizobial inoculation in relation to the density of indigenous rhizobial populations.

Robert F. 1991. A selective medium for the enumeration of wild type rhizobia in tropical soils by immunoblotting.

Symposium on Biotechnologies and Environment for a Sustainable Development. September 23-26, 1991, Montreal, Canada.

Somasegaran P. 1991. The NifTAL MIRCEN's contribution to international cooperation.

International Travel

1990

Abaidoo, Robert
Ghana
August 31-September 01, 1990

Bohlool, B. Ben
Kenya, Bulgaria
February 22-March 11, 1990

Bohlool, B. Ben
Thailand, Philippines
July 28-August 04, 1990

Bohlool, B. Ben
West Germany, Nigeria
September 17-October 03, 1990

Ferguson, Princess
Thailand
March 23-April 08, 1990

Hoben, Heinz
Morocco
April 13-June 15, 1990

Keyser, Harold
Haiti
February 28-March 09, 1990

Keyser, Harold
Uganda
November 14-December 03, 1990

Rourke, Joseph
Zambia, Uganda
May 15-August 15, 1990

Singleton, Paul
Thailand
March 16-April 09, 1990

Singleton, Paul
Philippines
April 14-May 02, 1990

Singleton, Paul
Uganda
May 13-June 10, 1990

Singleton, Paul
Nigeria
September 19-October 06, 1990

Singleton, Paul
Thailand, Nepal, Philippines
November 15-December 20, 1990

Somasegaran, Padma
Thailand
March 21-April 07, 1990

1991

Bartolini, Marcelo
Australia
August 09-21, 1990

Boonkerd, Nantakorn
Alberta, Canada
August 24-September 15 1991

Caces, Maria Luz
Alberta, Canada
August 25-September 03, 1991

George, Thomas
Philippines
January 12, 1991 -

Hoben, Heinz
Thailand
May 23-June 30, 1991

Hoben, Heinz
Alberta, Canada
August 24-September 03, 1991

Keyser, Harold
Alberta, Canada
August 22-31, 1991

Keyser, Harold
Nigeria, Togo, Ghana
September 06-24, 1991

Rice, Doug
Alberta, Canada
August 22-31, 1991

Rourke, Joseph
Australia
August 09-21, 1991

Singleton, Paul
Alberta, Canada
August 16-31, 1991

Somasegaran, Padma
Thailand
May 23-July 05, 1991

Thies, Janice
Alberta, Canada
August 24-30, 1991

Distinguished Visitors

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

Rodrigo Arias, Area Representative for NFTA, Guatemala

J. Ayuk-Takem, Institue of Agronomie Research, Cameroon

Tina Billow, NASA

Herman Bothe, University of Cologne, West Germany

Chutip Chanaseni, Dept. of Ag. Ext. Rice & Field Crops, Bangkhen, Thailand

Y.S. Chauhan, ICRISAT, India

Jack Croucher, Appropriate Technology International

Joshua Daniels, Area Representative for NFTA, India

Alan DeVilligas, Columbia

Margalith Galum, Tel Aviv University, Israel

Dennis Garrity, IRRI, Philippines

Peter Gresshoff, University of Tennessee

Sitiveni Halapua, Tonga, Pacific Islands Development Program

Nabil Hegazi, Professor/Researcher, Faculty of Agriculture, Cairo University, Egypt

David Herridge, Tamworth, Australia

Marae Irata, Kiribati, Ministry of Works & Energy, Tarawa

Brian Jarvis, Massey University, New Zealand

D. Gareth Jones, University College of Wales, UK

Phillip Kabua, Republic of the Marshall Islands, Office of the Chief Secretary

Noel Kefford, Dean, CTAHR

Sukanya Kongngoen, Dept. of Ag. Ext. Rice & Field Crops, Bangkhen, Thailand

Chrisanto Lopez, Assistant Professor, West Visayas State University, Philippines

Tewani Lui, Vanuatu, Chairman, Public Service Commission

Jim McMaster, Australia, University of Canberra

Pamela Matson, Stanford

Fujio Matsuda, Director, RCUH

Roy Nishimoto, Director, HITAHR

Glenn Patterson, Appropriate Technology International

Ralph Riley, Stanford

Mike Sadowsky, Department of Soil Science, University of Minnesota

Douglas Sawaichi, Republic of Palau, Senate, Palau National Congress, Assistant clerk

E.L. Schmidt, Department of Soil Science, University of Minnesota

Ray Smith, Assistant Director, HITAHR

Fred Swader, USDA, Washington, D.C.

Paul Stangel, Director, IFDC, Alabama

Kaiarake Taburuea, Kiribati, Minister of Line & Phoenix

Kay Tamaribuchi, Director, University of Hawaii Foundation

Alan Townsend, Stanford

Doug Turner, Stanford

Fusi Vave, Fiji, Prime Ministers Office

Peter Vitousek, Stanford

Carol Volkman, NASA

George White, USDA, Beltsville

Project Personnel

SCIENTIFIC and TECHNICAL STAFF

Robert Abaidoo, Research Associate

Wendy Asano, M.S., Research Associate (through 9/90)

B. Ben Bohlool, Ph.D., Director, NifTAL Project (through 5/91)

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

Dulal Borthakur, Ph.D., Molecular Geneticist, University of Hawaii

Luz Caces George, Ph.D., Molecular Geneticist, University of Hawaii

Thomas George, Ph.D., Agronomist, Philippines

Geoffrey Haines, Research Associate

Heinz Hoben, M.S., Foreign Training Coordinator

Kevin Keane, B.S., Research Associate

Harold H. Keyser, Ph.D. Plant/Soil Microbiologist, Head of Research (5/91 -)

Megan Loomis, B.S., Research Associate

Kathy MacGlashan, B.S., Research Associate

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

Patty Nakao, B.S., Research Associate, Head of Communications Department (8/91 -)

Douglas Rice, Ph.D., Molecular Geneticist

Francoise Robert, Ph.D., Microbiologist, University of Hawaii (partial support)

Joseph Rourke, B.S., Research Associate, Inoculation Production Specialist (5/91 -)

Patricia Scott, Laboratory Assistant

Paul Singleton, Ph.D., Head of Research (through 5/91), acting Director, NifTAL Project (5/91-)

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

SUPPORT STAFF

Lionel Amoral Jr., Groundskeeper

Colleen Anderson, Clerk typist (6/90-8/90)

Ellen Bellrose, Clerk typist (9/90-10/90)

Thipabha Bhupanthong, B.B.A., Clerk typist (Thailand)

Ann Coopersmith, M.A., Librarian (10/90 -)

Sally Ekdahl, A.A., Information Assistant (temporary hire 1/90-3/90, 12/90-6/91, 8/91 -)

Princess Ferguson, B.P.A., Head, Communication Section (through 8/91)

M. Anna Gillies, Graphic Artist (through 6/90)

Susan Hiraoka, Clerk Typist (through 8/91), Project Specialist (9/91 -)

Pam Hoopii, Utility Person (through 8/90)

Debra Hughes, Graphic Artist (temporary hire 2/90-4/90) (6/90 -)

Mack Jones, Maintenance

Thongpoon Muisattra, Office Maid (Thailand)

Ade Nikaido, Account Clerk

Nartkamol Pawai, B. Econ, Secretary (Thailand)

Barbara Sedano-Parrish, Clerk typist (10/90 -)

Dotty Shepherd, Library clerk/clerk typist (through 6/90)

Ray Smart, Utility Person

Frank White, M.B.A., Project Manager (8/90-7/91)

Karean Zukeran, A.A., Project Manager (through 6/90)

GRADUATE STUDENTS

M.Sc. Graduates

Dan Turk

Tanya Thongnunui (Thailand)

Ph.D. Graduates

Paul Woomer

Janice Thies

Maria Luz Caces

Ph.D. Degree Candidates

Surya Tewari

Bruce Linquist

Tola Fadare

Veronique Lambert

Nikhat Parveen (Thailand)

M.B.A. Candidate

Marcelo Bartolini

VISITING SCIENTISTS

Janez Hacin (Yugoslavia-Slovenia)

Nesime Cebel (Turkey)

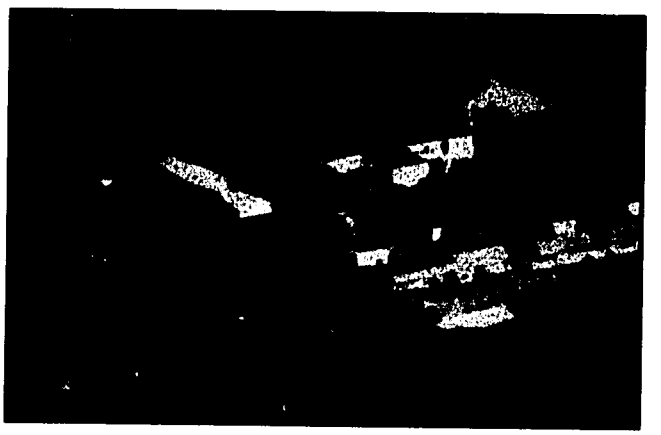
Andeas Wolff (Germany)

Remi Rakotondradona (Madagascar)

NifTAL Facilities

NifTAL headquarters are located on a 22.2 acre site at Hamakuapoko on the island of Maui, Hawaii. Hamakuapoko is the former site of the old Maui High School, built in 1900 and abandoned in 1970 as the population of the island shifted. The former school buildings used by NifTAL have been restored or maintained. Facilities at the headquarters include:

Administration wing - 1300 sq. ft.
Offices - 2,500 sq. ft.
Biotech laboratory - 1300 sq. ft.
Two microbiology laboratories - 2,200 sq. ft. total
Agronomy-soils laboratory - 1,300 sq. ft.
Media preparation area - 1000 sq. ft.
Plant growth room - 400 sq. ft.
Rabbit facilities - 400 sq. ft.
Dark room - 400 sq. ft.
Library-Communications building - 2,700 sq. ft.
Ben Bohlool Activities Center - 1,770 sq. ft.
Green House - 2,400 sq. ft.
Storage - 2,500 sq. ft.
Three trainee housing units - 2,500 sq. ft. (total)
Tractor shed - 5,350 sq. ft.



NifTAL headquarters at Hamakuapoko

One of these buildings, the Ben Bohlool Activities Center, has been renovated as a trainee conference center and includes a living quarters. Dr. B. Ben Bohlool was instrumental in restoring this facility, recognizing a need for meeting facilities for Pacific Islanders and other groups. In August, 1991, this building was dedicated in his honor as a researcher, leader, and for his many contributions to mankind.

In addition to NifTAL's main facilities, there is a 10 acre field site at Hamakuapoko which is reserved for training exercises, plant holdings, germplasm increase, and perennial experiments such as the seed production and spacing trials with *Leucaena*. There are also three other field sites on Maui.

One of these sites is Kuiaha, a 10 acre field with low phosphorus, highly acidic soil conditions (4.5 pH) that are considered a resource for studies on soil stress interactions with BNF. This site is also used for experiments on low phosphorous tolerance and other fertility management trials.

A 10 acre field site at Pauwela is used as a nitrogen fixing tree arboretum and for studies on the management of nitrogen cycling in cropping systems. Other projects at this site include a *Leucaena* seed production orchard. The pH at this site is 5.5.

The Haleakala field site differs from the other sites in that it is located at a higher elevation (660 M), has a cooler temperature, a pH ranging from 3.9 - 4.8, and is well suited for testing the cold tolerance of trees and other legume crops and their compatible rhizobia. This allows NifTAL to research the legume/*Rhizobium* combinations for tropical uplands. This 37.64 acre site also houses a two-bedroom, 1000 sq. ft. guest house, microbiology laboratory, greenhouse, and a self-contained pilot inoculant production plant.

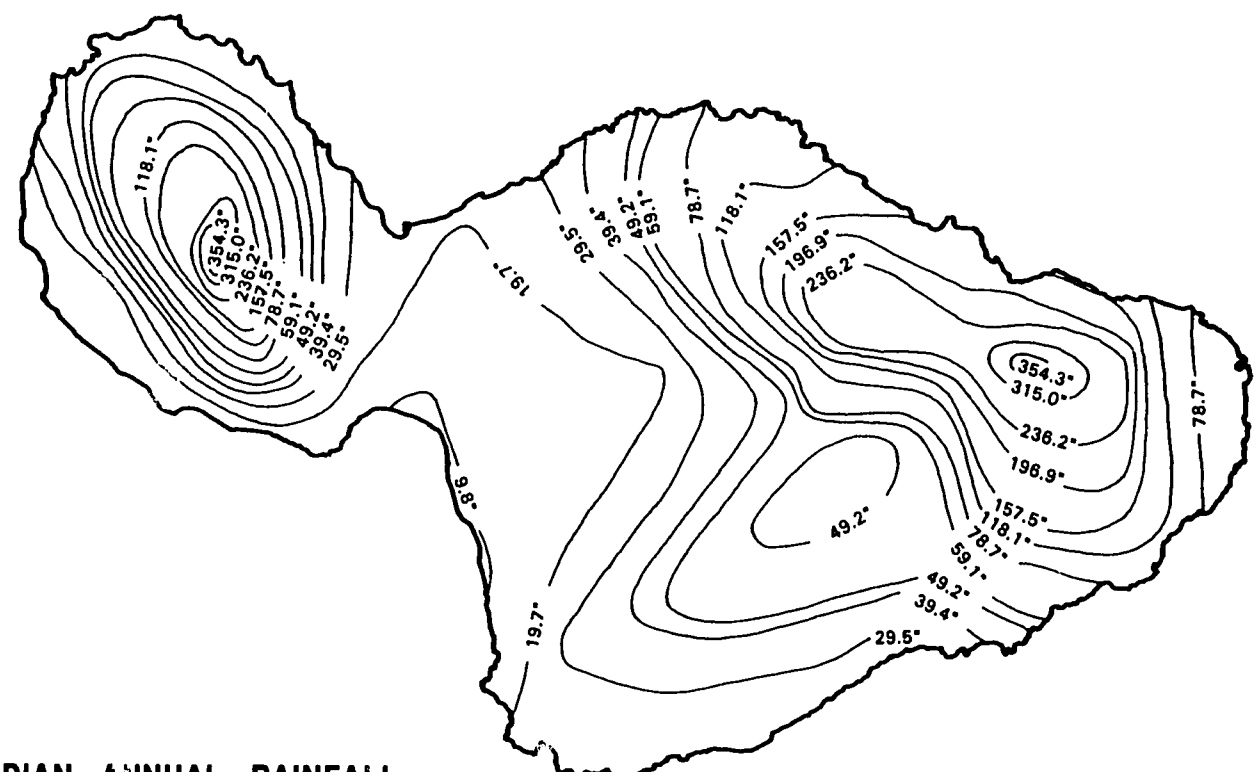
The MauiNET: A Living Laboratory

Many of the environmental conditions prevalent in the developing tropics can be found on the island of Maui. In addition to NifTAL's main field sites, the Project participates in the Maui Climate and Land Use Network, or MauiNet, which exploits the environmental diversity along the slopes of Maui's Mount Haleakala.

Haleakala is a massive shield volcano which rises to over 3000 M in elevation. Its slopes intercept the prevailing north-east tradewinds, resulting in orographic rainfall patterns and distinct climatic and vegetation zones. Within a compact area, vegetation varies from typical Hawaiian rainforests on the windward slopes, to xerophytic shrublands of the leeward shores, to montane and subalpine zones near Haleakala's summit. The environmental diversity patterning the slopes of this volcano creates an ideal "living laboratory" for ecosystem and agro-ecosystem studies.

The MauiNet consists of over 20 sites along two elevational transects (wet and dry) on the western slope of Haleakala. The transects extend from 40 to 1646 M in elevation, and 12 sites are currently equipped with automatic weather stations collecting data on air and soil temperatures, precipitation, solar radiation, relative humidity, and in some cases, wind speed and direction. The sites are situated in various morphogenetic, climatic, and vegetation zones. They offer an array of rainfall, temperature, and soil type regimes for the development of crop and microbial ecology models.

The MauiNet has been critical to NifTAL's development of ecologically based models describing the occurrence of native rhizobia and the persistence and symbiotic performance of introduced rhizobia in tropical environments. Many of NifTAL's scientific papers published in the previous four years are based on MauiNet resources. This living laboratory is one of NifTAL's advantages for conducting field studies applicable to tropical conditions.



MEDIAN ANNUAL RAINFALL
ISOHYETS IN INCHES

Training Course and Workshop Participants

Hawaii - "Modeling N-fixation for Grain Legume Crops" March 15-17, 1990.

Gordon Tsuji
Goro Uehara
Garrit Hoogenboom
James Jones
Paul Singleton
Paul Woomer
Janice Thies
Thomas George
Robert Caldwell
B. Ben Bohlool

Thailand - "International Training Workshop in Applied BNF Technology for Extension Specialists" March 26 - April 5, 1990. Sponsored by Secondary Food Crops Development Project, government of Indonesia, and United States Agency for International Development.

Ir. Maryam Amiruddin, Indonesia
Irdam Anwar, Indonesia
Ramlah Arief, Indonesia
Jacob Billik, Indonesia
Husni Fahri, Indonesia
Sutopo Ghani Nugroho, Indonesia
R. Mangitua H. Manurung, Indonesia
Donna Purba, Indonesia
Sri Daryasih Ratmo, Indonesia
Ir. Sadono, Indonesia
Agustinus Slamet, Indonesia
Herman Suheri, Indonesia
Achmad Sutardjo, Indonesia
Azran Tanjung, Indonesia
Ir. Zuraida, Indonesia
Mr. Saree Supametee, Thailand
Mr. Worawich Rungrattankasin, Thailand

Morocco - "Techniques in Rhizobiology of Pasture and Forage Legumes" April 22-May 5, 1990. Participants sponsored by FAO and ICARDA.

Lakhdar Chaouche, Algeria
Hafida Akrouf, Algeria
Mohamed Larbaoui, Algeria
Abdel S. El Desouki, Egypt
Taha Ahmed Mahmoud, Egypt
Iman A. Fergani, Libya
Muftah Mayub, Libya
Imane Thami Alami, Morocco
Mohamed El Khadir, Morocco
Mustafa El Adardor, Morocco
Mohamed Harkousse, Morocco
Majid Youssef Mezni, Tunisia
Ben Jeddi Faycal, Tunisia
Abdelkhalek Barbouche, Tunisia

Uganda -"Applied BNF Technology - A Practical Guide for Extension Specialists" November 26-30, 1990. Sponsored by Uganda Cooperative Alliance - Human Resources Development Department.

Sarah Sanyu Mujunya, Mityana
Nanyonjo Noelina, Mubende
Emmanuel Tumwizere, Mityana
Ssebbaale Edrisa, Lumero
Kigongo Gerald M. W., Kiboga
Kabuye S., Kampala
Kalulu P., Luwero
Ocheni J. Stephen, Luwero
Kiberu Katongole Francis, Kiboga
Immaculate Naggayi, Mityana
Kibwami A., Mpigi
Katende V., Mityana
Brosa Christopher, Luwero
Oluka-Akileng I., Kampala
Kwesiga Rweenda Frank, Kampala
Kizito P., Kampala
Speciosa Namusisi, Mityana
Patrick Buyinza, Kampala
Candia R., Mpigi
Francis Gonahasa, Kampala
Kagumaho Emmanuel, Kampala

Thailand - "Modern Methods for the Study of Rhizobia" June 3-28, 1991. Co-sponsored by the International Atomic Energy Agency (IAEA), BNF Resource Center, Thai Department of Agriculture and the NifTAL Project.

Ajit Kumar Podder, Bangladesh
Chen Ming, People's Republic of China
Wang Huixian, People's Republic of China
Anisah Bt. Husain, Malaysia
S. Subasinghe, Malaysia
James Clugston, United States
Marly Silver, Uganda
Nguyen Ngoc Quyen, Vietnam
Khairuddin bin Abdul Rahim, Vietnam
Shaheen Asad, Pakistan
Crispina M. Rosales, Philippines
Edwin DEL Rosario, Philippines
R. Senaratne, Sri Lanka

"Indo-U.S. Science and Technology Initiative" September 16-18, 1991. Sponsored by the United States Agency for International Development.

Y.D. Gaur, India
N.N. Goswami, India
K.V.B.R. Tilak, India
M.C. Sarkar, India
B.D. Kaushik, India
Charles Lindau, United States
K.R. Reddy, United States
Dennis Rolston, United States
William Piekielek, United States
Richard Fox, United States
John Duxbury, United States
Sandra Nierzwicki-Bauer, United States
Barbara Kittell, United States
D.W. Rains, United States
Sharon Stanfield, United States
Sham Goyal, United States
Paul Singleton, United States
Padma Somasegaran, United States

NifTAL Receives External Review

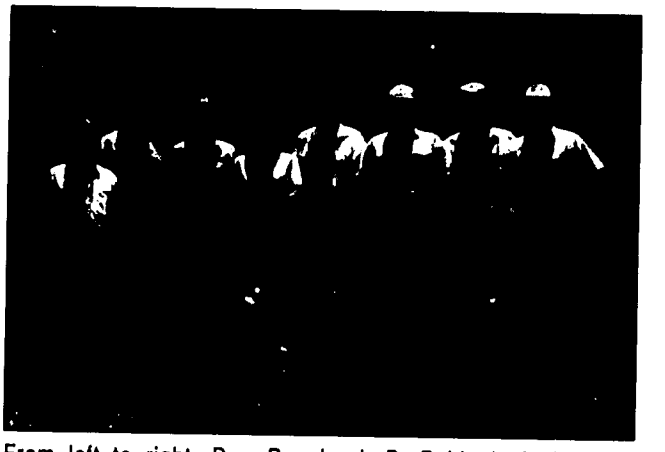
In 1986 AID entered a cooperative agreement with the University of Hawaii to enhance its resource base in international BNF through the NifTAL Project. At that time, NifTAL was envisioned as a ten year project to conduct research, training, and outreach activities promoting BNF. Part of the cooperative agreement entailed triennial external evaluations to assess the performance and impacts of NifTAL's programs in meeting these goals.

In August, 1990, NifTAL hosted the review panel of the first triennial external evaluation. This distinguished panel was led by Dr. John Nickel, former Director General of the Centro Internacional de Agricultural Tropical (CIAT). The other members were Dr. John Anderson, Production Economist at the University of Georgia; Dr. David Bezdicek, plant and microbial BNF researcher from Washington State University; Dr. Ann Hirsch, BNF molecular biologist from University of California, Berkeley; and Dr. O.J. Reichman, Director of the Ecology Program at the National Science Foundation.

Prior to the NifTAL site review, Dr. Nickels, accompanied by Project Director Dr. Ben Bohlool and AID Program Officer Dr. Charles Sloger, visited the BNFRC in Thailand. There the review team leader evaluated the positive influences of the BNFRC on Thai crop production and on BNF activities in the South East Asian region. Following this visit, Dr. Nickels visited the Philippines to determine NifTAL's impact on the country's BNF research and development activities, and to review NIfTAL's collaborative projects with Philippine national and international agricultural institutes.

The entire review team later visited NifTAL headquarters on Maui and NifTAL's Biotechnology Program at the University of Hawaii, Manoa Campus. They had the opportunity to assess NifTAL's staff, facilities, accomplishments, as well as the future challenges facing the project.

In their overall assessment, the review team concluded, "NifTAL is a productive, well-managed project, with dedicated staff and effective leadership. The project deserves continued support by AID. It is conducting high quality research and providing a valuable service to developing countries in an area of great relevance to sustainable agricultural development and agroforestry. NifTAL plays a unique role in covering a broad spectrum of BNF activity." The team provided further detailed evaluation of the project, as well as positive and constructive recommendations for its future.



From left to right, Drs. Boonkerd, B. Bohlool, J. Allison, A. Hirsch, J. Nickels, O.J. Reichman, D. Bezdicek, and C. Sloger during NifTAL review.