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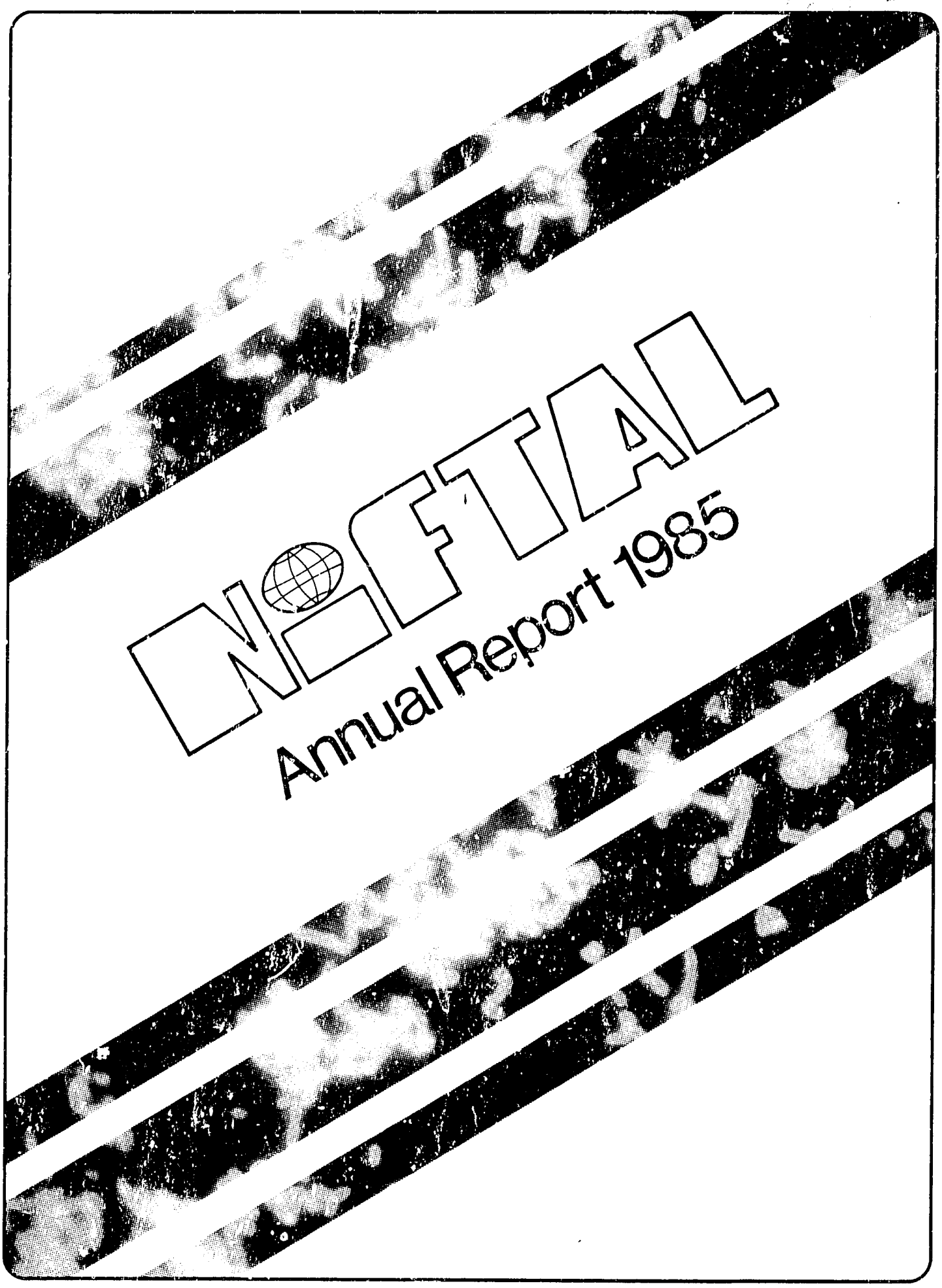

NITROGEN FIXATION

BY TROPICAL AGRICULTURAL LEGUMES

NIFTAL
UNIVERSITY OF HAWAII/USAID

ANNUAL REPORT 1985

USAID Contract No. DAN-0613-C-00-2064-00
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UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT

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SECTION I: SYNOPSIS

STATISTICAL SUMMARY

Title	: Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL Project)
Contract Number	: DAN-0613-C-00-2064-00 (was AID/ta-C-1207)
Project Number	: 931-0613
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Contractor	: University of Hawaii Department of Agronomy and Soil Science College of Tropical Agriculture and Human Resources 3050 Maile Way Honolulu, HI 96822
Contract Period	: 06/16/82-12/31/86
Reporting Period	: 01/01/85-12/31/85
USAID Funds to Date	: $4,225,000
Current Year Expenditures	: 500,000
Estimated 1986 Funds	: 650,000
Total Contract Funds	: 4,875,000
Project Office	: S&T/AGR/RNR
Project Monitor	: Dr. Lloyd R. Frederick

GENERAL BACKGROUND

The NifTAL Project was established in 1975 at the University of Hawaii under contract to USAID, additional funding has been provided from NSF, NAS, Unesco, USDA, the Science Advisor's Office-AID, the Donner Foundation, and the Rockefeller Foundation as individual grants to NifTAL researchers.

The focus of the NifTAL Project is a process (biological nitrogen fixation) rather than a commodity or a discipline. In fact, NifTAL rallies the expertise of many disciplines to address a process which benefits an array of commodities.

Some constraints on fuller utilization of biological nitrogen fixation are technical and are being addressed through research. Economic and social factors also limit research, development, and use of inoculants.

PROJECT GOAL

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

Achievement of this goal required that NifTAL institute the following activities:

1. Backup support to LDC BNF workers. NifTAL provides support by supplying research quantities of inoculant; pure cultures of *Rhizobium*; strain specific antisera to identify strains of *Rhizobium*; consultation, by mail and in person, on all aspects of legume inoculation; reprints of journal articles not readily available to LDC BNF workers; and by expediting communications and information flow among LDC BNF workers; and by expediting communications and information flow among LDC BNF workers, etc. Backup support generally does not entail monetary support which is provided through USAID country missions.

2. Coordinates worldwide legume inoculation trials in the tropics. These trials provide information which, in addition to being of value to the LDC scientists themselves, is useful to donor agencies and host country policy makers in assaying requests for funding legume inoculation activities. They also insure that all LDC legume inoculation workers have quality inputs in terms of valid experimental design, high quality inoculant, materials for strain identification, and proper statistical analysis.

3. Conducts research directly relevant to BNF problems in the tropics. Research, although primarily conducted at the Hawaii headquarters, is aimed directly at problems that exist in the LDCs or which can be anticipated in the short term future.

4. Provides training in all aspects of legume inoculation technology to BNF workers in the tropics. Such training is provided by a combination of training courses held in Hawaii, training courses held in host country institutions, individualized training for advanced workers in Hawaii, and graduate degree training.

ACKNOWLEDGEMENTS

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

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

SUMMARY OF ACCOMPLISHMENTS

The year 1985 saw a continuation of the many efforts undertaken by NifTAL.

Experiments were conducted in many areas. Research topics included comparing methods for measuring N-fixation; assessing competition for soil nitrogen; studying the effect of N-yield on percent ^{15}N using a non-fixing reference plant in the ^{15}N dilution method; looking at the mechanisms controlling competition for nodule sites and development; observing the interaction between environment, physiological and N-fixation and between soil fertility and inoculation rate on soybean; studying noduation and fixation in N-fixing trees; assessing the effect of inoculation rates on N-fixation and competition for nodulation in chickpea; comparing the impact of different soil factors, rhizobia, and organic phosphate on rhizobia nutrition; characterizing salinity on three major legume species; and conducting an on-farm study of BNF economy in legume/rice-based rotations in Thailand.

The Rhizobium Germplasm Resource has been over 60% lyophilized. This process ensures long-term storage and facilitates transportation of the more than 1700 strain collection. The number of 1985 requests for cultures increased over 1984 by 142% and the number of distributed cultures increased by 197%. Arrangements were made to transfer the NifTAL culture collection in lyophilized ampules to IARI, Department of Agriculture, to form the basis of their culture collection. Transfer is also planned for the computer data that support the collection. Requests for antisera also increased with a total of 374 ml supplied to researchers in 16 countries.

Scientists from developing countries have learned to use NifTAL's medium scale, low-cost fermenter system for mass culturing of Rhizobium. This system should prove to be an efficient and appropriate way for developing country inoculant producers to make quality legume inoculants.

The International Network of Legume Inoculation Trials has 197 cooperators in 46 countries. During the year, 34 countries were serviced with inoculant, "A" experiments, cultures, and/or seeds. A bulletin published this year included information on preliminary results which indicate that a response often can be obtained from inoculation with proper Rhizobium. A secondary achievement of the INLIT has been identification of research workers in the tropics interested in legume inoculation, convincing them of the advantages of conducting uniform field trials, and improving the quality of research work and research worker's expertise by providing support services.

The BNF Resource Center in Bangkok continued to offer assistance to BNF workers throughout the South and Southeast Asian area. In addition to offering a wide variety of services, an extension training course was offered to 25 participants, in which extension and research workers learned the basics of legume inoculation.

Another NifTAL-offered training course was also directed toward extension workers. This course was offered to 23 persons in Bangladesh. NifTAL also participated in a 4-week Rhizobium technology course offered to 17 Latin American scientists at the Porto Alegre, Brazil, MIRCEN. A seminar cosponsored by NifTAL was the N-fixation seminar for Central American BNF workers held in Honduras. Other training participants during the year included six graduate assistants studying at the University of Hawaii and three visiting scientists interning at the NifTAL site for from 5 to 6 months each.

NifTAL met the challenge of hosting the 10th North American Rhizobium Conference by incorporating other meetings into the Conference. A BNF Limiting Factors Workshop included 60 participants; BOSTID held its annual workshop for recipients of NAS/CGR N-Fixation grants; the U.S./Japan Seminar on Factors Limiting Nitrogen Fixation by Soybeans met; and several regional meetings were informally convened among conference participants.

Highlights of this year's outreach activities included cooperation with the Zamare Project in Zambia and the Indo/US science and technology initiative. With Zamare, a NifTAL technician was key in the production of 3,473 Kg of quality soybean inoculant--this was a 300% increase over 1984 production. This resulted in a significant increase in crop production. The areas of emphasis in the Indo/US cooperation included studies directed at improving production and quality assurance of Rhizobium inoculants, management practices that increase BNF, and serology and genetics of Rhizobium for soybean and chickpeas.

NifTAL research output was disseminated through journal publications and conference/workshop presentations. Nearly 1,300 requests for scientific reprints and NifTAL publications were filled and 1,700 readers in 100 countries continued to receive the BNF BULLEIN newsletter. Major publications included an antisera catalogue and a 390-page laboratory manual.

It was a challenging year for NifTAL's staff as they continued to meet the objectives of the project through research, outreach, and training activities.

SECTION II: ACCOMPLISHMENTS

INTERNATIONAL NETWORK OF LEGUME INOCULATION TRIAL

Objective

The primary objective of the International Network of Legume Inoculation Trials (INLIT) is to determine whether under definitive conditions a yield increase can be obtained through legume inoculation using the present State of the Art.

Two additional crops--*Phaseolus lunatus* and *Psophocarpus tetragonolobus*--were included in the INLIT program for testing.

Transferred Network Coordination for the Asian Region to the BNF Resource Center in Bangkok. All requests from India, Pakistan, Burma, Nepal, Sri Lanka, Philippines, Malaysia, and Indonesia were serviced by that Resource Center.

Published a bulletin through the University of Hawaii, College of tropical Agriculture and Human Resources, "Design & Analysis of an International Experimental Network: Legume Inoculation Trials in the NifTAL Project, the INLIT Experience." This bulletin reviewed the history of the trials, need for them, formulation of their actual components, and their general objectives. Preliminary results indicate that a response often can be obtained from inoculation with proper *Rhizobium*. Responses have even been noted with some traditional crops where the presence in the soil of *Rhizobium* of the correct cross-inoculation group would be expected.

Also achieved have been the secondary objectives of identifying all research workers in the tropics interested in legume inoculation, convincing them of the advantages of conducting uniform field trials, and improving the quality of research work and expertise of research workers by providing support services.

Asian Network

Bangladesh: Made an assessment of the BNF situation in the country and made recommendations to the Bangladesh Agricultual Research Council (BARC) for a coordinated country-wide program. Served as main lecturer and assisted in conducting the second BARC-NifTAL Extension/research BNF course which was held at the Graduate Training Center, Bangladesh Agricultural University, Mymensing.

Pakistan: Worked with Pakistan Agricultural Research Council (PARC) BNF Coordinator to make an assessment of BNF activities at the Provincial Research Institute, served as advisor to the BNF Program at the National Agricultural

Research Center (NARC), and planned the fabrication and installation of a pilot legume inoculation plant at NARC.

Thirty-four countries were serviced by the INLIT programs sending out requests for inoculant, "A" experiments, cultures, or seeds:

Argentina	Haiti	Peru
Australia	India	Philippines
Bangladesh	Indonesia	Rwanda
Bolivia	Kenya	Saudia Arabia
Brazil	Madagascar	Sudan
Burma	Malaysia	Togo
Colombia	Mali	Uruguay
Dominican Republic	Mexico	Venezuela
Ecuador	Niger	Sierre Leone
El Salvador	Nigeria	Zaire
Ghana	Pakistan	Zambia
Guatemala	Panama	

There are presently 197 cooperators in 46 countries (Appendix I).

ZAMARE PROJECT

ZAMARE Project - University of Illinois/NifTAL/USAID Zambia Mission

NifTAL has provided a microbiologist and an inoculant production specialist to the Mount Makulu Research Station at Lusaka, Zambia, under a subcontract from the University of Illinois. The Illinois ZAMARE Project (Zambian Agricultural Research and Extension) is a multi-disiplinary project, an aspect of which involves soybean improvement and production programs. NifTAL has provided technical assistance in research, production, and distribution of Rhizobium inoculants to meet expanding production of soybean and other legumes in Zambia.

Through NifTAL participation, production of inoculants at the Mount Makulu facility was increased by 300% over the 1984 total. This amounted to 3,473 Kg of soybean inoculant. Quality control protocol instituted at the facility indicates that the inoculants have in excess of 1×10^9 viable rhizobia per gram. Zambian farmers have been extremely successful in establishing in their soils the rhizobia carried in these inoculants. Current inoculation rates per hectare are low due to insufficient inoculant supply, however, farmer successes attest to the high quality of inoculant being produced. Information and instruction on transport, storage, and application of the inoculants also contibuted to the high success rate in the field.

For the first time, all inoculants were produced using peat mined and processed in Zambia. Tests performed earlier in the subcontract had shown these peat deposits to be of excellent quality for inoculant production. The inoculants were produced in sterile (autoclaved) peat using a broth dilution technique since large scale fermentation capability is not currently available at Mount Makulu. Tests were also initiated in 1985 to assess the suitability of Zambian peat for use as a non-sterile carrier material. In addition, plans were made to obtain low cost, reliable fermentation equipment that will provide the facility with an alternate inoculant production technique in the future.

Research efforts continued in 1985 and confirmed earlier preliminary experiments concerning the response to inoculation of soybean in Zambia. Field trials at diverse sites in soybean growing regions of Zambia indicated that soybean responds significantly to inoculation with Rhizobium. Sites of various field tests included Nanga, Golden Valley, Mufulira, and Msekera. The inoculation trials always included cultivars from North American and Zambian cultivars bred to nodulate with native rhizobia. This nodulation characteristic was incorporated into local cultivars to eliminate the need for inoculation by small farmers in Zambia. Many experiments also contained two management variables designated Farmer Practices and High Management Treatment (blanket applications of P, lime, K, Mg, and Mo). Nitrogen controls were included to measure the N input required to meet the yield obtained with inoculation. Results are summarized in Appendix II, Table 1. Inoculation with effective strains of Rhizobium japonicum always resulted in increased yields.

The response shown is relatively greater at sites where there had been no previous soybean cultivation such as the plots used at Golden Valley. Responses to inoculation were generally greater in magnitude where yield potential was raised with fertilization. The Zambian cultivars generally yielded more than North American cultivars when no inoculum was applied. However, Zambian cultivars consistently responded to inoculation with Rhizobium japonicum. Yields indicated that inoculation can provide nitrogen to soybean in Zambia equivalent to a fertilizer N application of 100-200 Kg N/ha^{-1}. Inoculation is strongly recommended for all farmers. Those farmers who cannot obtain inoculum, however, should plant Zambian cultivars.

A preliminary experiment was conducted to help explain inoculation results in terms of Rhizobium population dynamics in Zambian cropping systems. Soil samples were collected from three fields at Mkushi. The samples were taken from: 1) non-cultivated; 2) previously planted with uninoculated soybean in 1984; or 3) planted with inoculated soybean in 1984. The number of rhizobia present was determined by the most probable number (MPN) plant infection

technique using three hosts to sample the population of rhizobia (Appendix II, Table 2). Counts with mungbean determine the general native population of cowpea-type rhizobia. Hernon soybean was bred to nodulate with native rhizobia while soybean cultivar Kaleya is more resistant to nodulation by native rhizobia and resembles North American cultivars for this characteristic.

Results indicate that counts on Hernon and mungbean in non-cultivated soil were similar while there were very few native rhizobia available for Kaleya. Planting soybean without inoculation enriched the soil for rhizobia nodulating Hernon and Kaleya but not mungbean. Previous inoculation enhanced numbers for both soybean cultivars above the native population.

These results may have implications for the need to inoculate in fields under repeated soybean cultivation, but this assumption requires additional research. It appears that the native rhizobia nodulating Zambian cultivars are a subset of the native cowpea rhizobia population and are specifically increased through soybean culture.

INDO/US SCIENCE & TECHNOLOGY INITIATIVE

The areas of emphasis in this program include studies directed at: 1) Improved production and quality assurance of Rhizobium inoculants; 2) Environmental conditions and management practices that increase BNF in pigeon pea and soybean systems; and 3) Serology and genetics of Rhizobium for chickpeas and soybeans.

The potential for using beneficial "helper organisms" combined with rhizobia in peat inoculants is being investigated. "Helper organisms" are micro-organisms which may improve the quality of inoculant by improving shelf life under adverse storage conditions, stimulating rhizobial growth within the peat, stimulating root development on the host plant, or protecting nodules from insect damage. Organisms under consideration include Lipomyces, Penicillium, Azospirillium, Bacillus, and Pseudomonas species. Initial experiments have demonstrated compatability of these organisms with Rhizobium cultures when incubated in sterile peat.

Some strains of Rhizobium for chickpeas have proven to be poor immunogens, in that conventional injection procedures have yielded poor quality rabbit antiserum for serological work. In collaboration with scientists at Indian Agricultural Research Institute (IARI) and ICRISAT, we have been successful in producing improved antisera, for agglutination test and immunofluorescence, by using the intra-footpat route of injection in rabbits.

GERMPLASM

Objective:

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

Approximately 61% of the NifTAL culture collection has been lyophilized by the end of 1985. We expect to complete lyophilization of the collection by the end of the NifTAL contract. This process will ensure that our collection of over 1700 strains will have proper long-term storage.

In 1985, NifTAL sent out 1,151 cultures in response to 104 requests coming from 34 countries. (See Appendix III.) While the number of requesting countries remained the same as 1984, the number of requests increased by 142% and the number of cultures distributed also increased by 197% compared to 1984 figures.

INOCULANT PRODUCTION

Objective:

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

Following the Inoculant Production Training Course which was held at NifTAL in December, 1984, the low cost stainless-steel fermenters for mass culturing of Rhizobium were further tested and improved. They yielded very promising results and it is strongly felt that this NifTAL-designed system is appropriate for developing countries. The fermenters are autoclaves in themselves and can be fueled by gas or charcoal for the sterilization of the growth medium. Fermenters with capacities of 141 litres have been successfully tested. Visting scientists from developing countries have succeeded in using the systems to grow rhizobia during their internships at NifTAL.

ANTISERA DEVELOPMENT

Objective:

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

The NifTAL Rhizobium antiserum bank now holds antisera for the identification of all INLIT strains. Details are shown in list A of the NifTAL antisera catalog. A separate group of miscellaneous antisera is shown in List B of the catalog. These antisera are available to researcher until the supply is depleted.

Plans are under way to produce antisera at NifTAL's Rhizobium Resource Center in Bangkok, Thailand-Production will start next year.

An antisera production facility for the purpose of teaching and research is in the planning stage. Construction will start at NifTAL's Haleakala site when funds become available.

A compact manual describing the production and use of antisera is near completion. It's publication is subject to the availability of funds.

Requests for antisera have increased in number over the past year. A total of 374 ml were supplied to researchers in 16 countries (See Appendix IV).

INFORMATION

Objective:

To disseminate NifTAL reserach output and transfer technical information for research, extension and production enterprises required to assess and use technologies based on BNF for crop production in the tropics.

NifTAL's research output is disseminated through journal publications and conference/workshop presentations. See Appendix X (Bibliography 1976-1985) and Research Abstracts following the Research accomplishments.

During 1985 there were 150 requests for scientific reprints authored by NifTAL staff and more than 1,100 requests were filled for other NifTAL publications.

Technical information on biological nitrogen fixation is also available to researchers in developing countries through the NifTAL document collection. The 8,300-document collection contains both current and classic articles. In 1985, 500 documents were added to the collection. The periodic bibliography distributed with the BNF BULLETIN continues informing researchers of articles and documents available free of charge to developing country BNF workers. NifTAL serviced more than 250 requests for multiple documents from researchers in 40 countries.

NifTAL's BNF BULLETIN newsletter continues to be sent to 1,700 readers in 100 countries. Three issues were distributed during the year. The newsletter sustains awareness by an international development audience of training courses, general BNF information, and projects in progress worldwide that impact on the use of BNF in agriculture. The BULLETIN is also a vehicle for exchange of ideas between researchers and decision makers.

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

Publications produced included the 390-page laboratory manual "Methods in Legume Rhizobium Technology" by Padmanabhan Somasegaran and Heinz Hoben. The book was field tested and perfected at NifTAL's many training courses. Also produced was the 16-page booklet "NifTAL Antisera Resource Catalogue." Other publications were the 10th North American Rhizobium Conference Book, 4 anouncements, and reprinting of the booklet "Resources for Development." Several scientific poster presentations were prepared for the 10th North American Rhizobium Conference. Audio-Visual productions were a revision of the "What is NifTAL" slide-tape and specialized slide sets for meeting presentations.

TRAINING

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

As in previous years, NifTAL continued offering training opportunities for professionals in the form of graduate assistantships in M.S. and Ph.D. programs, short-term internships at NifTAL Headquarters on Maui, and specialized training courses in Rhizobium technology.

Graduate Assistantships

Six graduate assistants have been studying at the University of Hawaii under NifTAL guidance and under full or partial NifTAL sponsorship during 1985.

Tafiqul Aziz (Bangladesh) and Thomas George (India) are pursuing a Ph.D. degree at the Department of Agronomy and Soil Science. Aziz is investigating the effect of soil erosion and inoculation with WMA fungi and rhizobia on the N-fixation of Leucaena and *Sesbania* spp. George is looking at the effect of elevation on the physiological development and N-fixation of soybean.

Steven Dowdle (USA) completed his Ph.D. studies at the Department of Microbiology in June. The title of his dissertation was "The ecology of *Rhizobium japonicum* in soybean-rice cropping systems in Central China."

Naseer Mirza (Pakistan), Ronnie Nyemba (Zambia), and Wilson Kagabo (Liberia) are studying for their master's degrees at the Department of Agronomy and Soil Science. Mirza's research will be conducted on Maui where he will study the effect of soil moisture and temperature on the establishment of agricultural soils on Maui.

Nyemba is looking at the interaction between inoculation rate, P-fertility, and N-fixation by soybean. His program is financed by the Zamare Project (University of Illinois).

Kagabo is investigating the nitrogen requirements of field grown legumes as measured by the N^{15} isotope dilution technique and nitrogen balance methods. His work is supported by a USAID loan to Liberia (University of Louisiana and University of Missouri).

Interns

During 1985, scientists from Indonesia, Turkey, and The Philippines served extended internships.

Muhrizal Sarwani (Selantan, Indonesia), an agronomist specializing in field crops of tidal swamp areas, attended the NifTAL inoculant production course in December 1984. After the course, he stayed on at NifTAL Headquarters for a five-month internship during which he worked on the following greenhouse research projects.

In collaboration with Padmanabhan Somasegaran and Heinz Hoben, he participated in an experiment to assess the host-strain interaction between cultivars of *Phaseolus vulgaris* and *Rhizobium phaseoli*.

He further collaborated with Paul Singleton in two experiments. First, they investigated the symbiotic action of Rhizobium japonicum and Bradyrhizobium japonicum with cultivars of soybean, and, second, they assessed the effects of lime, zinc, and molybdenum on N_2-fixation by soybean.

Dr. Velittin Gurgun, Associate Professor in the Department of Agricultural Products, University of Ankara, Turkey, also spent 5 months at NifTAL. During his stay, he worked with P. Somasegaran and H. Hoben on two experiments.

In one experiment, he studied host-Rhizobium interaction in the Kabuli and Desi cultivars of chickpea using ten Rhizobium strains. The other was a pot experiment in which the influence of five inoculation rates was investigated using two chickpea cultivars and two soils. In this experiment, three antigenically distinct strains of Rhizobium represented in equal numbers were used in a peat-based inoculum and the strain competition was also studied.

Rolando O. Torres, a researcher of the International Rice Research Institute, Los Banos, Philippines, also completed a 5-month internship at NifTAL. The main objective of his visit was to learn techniques and methods in Rhizobium technology which are applicable to his research on N-fixation in multiple cropping systems.

Torres learned these techniques while participating in two pot experiments with P. Somasegaran and H. Hoben. The first was a study on competition and survival of Rhizobium japonicum in soil inoculated with a 3-strain mixed inoculum applied by six inoculation methods. In the second experiment, host-Rhizobium interactions on four Phaseolus lunatus cultivars were studied using three different strains. In order to study strain competition, these were applied as a single-strain inoculum, a double-strain inoculum, and a triple-strain inoculum.

CIAT/Porto Alegre MIRCEN/NifTAL Course

A four-week training course in Rhizobium technology was held from 16 November to 16 December at the Department of Soil Science, Faculty of Agronomy, UFRGS, Porto Alegre, Brasil. The course entitled "Methods for Evaluating the Contribution of Rhizobium Inoculants to Productivity of Tropical Legumes" was sponsored by the United Nations Development Program (UNDP). It was jointly organized by CIAT, the Porto Alegre MIRCEN, and NifTAL. The participants were 17 scientists from nine Latin American countries and one African country. A detailed list of participants is included in Appendix V and a schedule of lectures and a course outline are shown in Appendix VI.

SUMMARIES OF RESEARCH

1 Nodulation and nitrogen fixation in a *Leucaeana leucocephala* x *L. diversifolia* hybrid.

Whenever two different species of legumes belonging to the same genus are crossed, it is important to examine the nodulation and N-fixation of the hybrids. It has been shown that *L. leucocephala* and *L. diversifolia* can be successfully hybridized and the resulting progenies perform well in acid soil compared to *L. leucocephala*. However, since no data is available on the nodulation and N-fixation traits of the hybrids, a preliminary experiment was done in Leonard jars to study the nodulation and N-fixation traits of this *Leucaena* hybrid with a variety of *Rhizobium* strains.

Results indicated that the nodulation and N-fixation traits of the hybrid were significantly different from *L. diversifolia* but not generally different than *L. leucocephala* (Appendix VII, Table 1). TAL 1145 (CIAT 1967) was superior in all the nodulation and N-fixing traits (Appendix VII, Table 2). TAL 1145 was the most competitive of the three strains occupying 86% of the nodules in the mixed inoculation treatment.

Results indicate that the hybrid *Leucaena* has greater N_2-fixation potential than *L. diversifolia* with standard *L. leucocephala* inoculant strains. These results suggest that a strain selection program for hybrids will not have to be undertaken.

2 Effect of *Rhizobium* inoculation rates on N-fixation and competition for nodulation in chickpea (*Cicer arietinum* L.)

In a factorial experiment, Kabuli and Desi types of chickpea were sown in two soils (Ultisol and Oxisol) and inoculated with four rates of peat based inoculant (10^1, 10^2, 10^4, 10^6, and 10^8 rhizobia per seed). The inoculant contained equal numbers of three antigenically distinct strains of chickpea rhizobia (TAL 480, 620, and 1148). Measurements of shoot dry weight and shoot nitrogen (Appendix VII, Table 3) showed significant differences for inoculation rate ($p = 0.001$). The interaction between inoculation rate and soil type were significant ($p = 0.01$) for nitrogen accumulation. The results also indicated that the inoculation rate and chickpea type interacted significantly ($p = 0.001$) for nitrogen accumulation in the shoot.

Strain identification in the nodules indicated that TAL 620 and TAL 1148 were close to being equal in their competitiveness irrespective of soil type or chickpea variety. TAL 480 was detected in very low frequency in the nodules in all treatments indicating that it was a poor competitor for nodulation. This study indicated that at least 10^4 rhizobia per seed were needed to achieve adequate nodulation and N-fixation in soil free of native chickpea rhizobia.

3 Measuring N_2-Fixation by Leucaena leucocephala under field conditions; a comparison of methods.

Measuring N_2-fixation by legumes under field conditions is difficult and cumbersome. Until now the methods available have not been tested simultaneously under field conditions and it is not know which method is the most reliable and practical. To test methods for measuring N_2-fixation by trees in the field, Leucaena leucocephala was planted under the following conditions: 1) soil free of Rhizobium that can nodulate L. leucocephala and no ^{15}N applied; 2) soil free of Rhizobium and ^{15}N applied; 3) inoculated with Rhizobium strain (TAL 1145) and ^{15}N applied; and 4) inoculated with TAL 1145 and no ^{15}N applied. These treatments permit comparison of N_2-fixation measurements of Leucaena by four methods: 1) the N-balance method; 2) the ^{15}N-dilution method; 3) ^{15}N-natural abundance method; and 4) ureide method. Results of this two-year experiment should provide information on which method of estimating N_2-fixation by a tree is most practical and accurate.

4 Ureide, amno-acids, and nitrate in trees: an indication for N_2-fixation.

N-compounds transported from the roots to the shoots of legumes are found to be in the form of nitrate, amino-acids, or ureides. It has been shown that ureides are predominately produced by nodules of some legumes whereas amino-acids can be produced by nodules as well as roots. Therefore, determining the amount of uriedes present in the xylem or leaf tissue compared to other N compounds may provide an indication about the proportion of N derived from N_2-fixation at a point in time.

The measurement of N_2-fixation by trees with conventional methods is difficult. The use of ureide measurements may prove useful with trees since extracts can be made non-destructively. The usefulness for estimating N_2-fixation by measuring ureides in xylem or leaves is being tested in 50 species of tropical leguminous trees. These trees are being grown in a greenhouse under two conditions: 1) N free, inoculated with Rhizobium and 2) excessive mineral N (NH_4NO_3) without inoculation. All nitrogen in the first treatment is derived from N_2-fixation while the second is completely dependent upon the assimilation of mineral N.

After a growth period of 5 months trees will be harvested and analyzed for nitate, ureides, and amino acids. Results will indicate which of the tested species produce sufficient ureides to make this procedure a reliable indication for measuring their N_2-fixation activity.

5 Competition for soil N between N_2-fixing and non-N_2-fixing soybeans.

While nodulated legumes have two sources for N, soil N, and atmospheric N_2, non-N_2-fixing plants can only assimiate mineral soil N. Through evolutionary processes the N_2-fixing plant may have lost some of its competitiveness for soil N uptake as compared with a non-N_2-fixing plant. To test this possible phenomenon, root systems of soybeans of two soybean plants were divided between three pots (see Figure). Pots were filled with Rhizobium japonicum-free soil. Pot A was inoculated with R. japonicum, pots B and C received mineral N at such levels that equal growth the N_2-fixing and non-N_2-fixing soybean was obtained. Four weeks after planting ^{15}N-labeled ammonium sulfate was applied weekly to pot b (pictured in the center of Figure). After four weeks of N application, plants were harvested and analyzed for atom percent ^{15}N. It appeared that the N_2-fixing and non-N_2-fixing plants were equal competitors for available soil N. Substituting effective Rhizobium strains for less effective ones did not change significantly the plant's capability to compete for available N.

6 Effect of N-yield on atom percent ^{15}N of corn used as a non-N_2-fixing reference plant in the ^{15}N isotope dilution method for measuring N_2-fixation.

Measuring N_2-fixation by the ^{15}N-dilution method requires the use of a non-N_2-fixing reference plant and is based on the assumption that the atom percent ^{15}N of the reference crop is independent of total N accumulation. This assumption was tested in two field experiments using corn as the non-N_2-fixing plant. In the first experiment, corn was grown at 9 different plant densities--6 densities as a monocrop and 3 densities intercropped with cowpea. Plants were harvested 50 and 80 days after planting. Results indicated that, for both harvest periods, significant differences in total N-yield between plant densities were observed but the atom percent ^{15}N remained constant.

In a second experiment plant densities were constant but plant yield varied with four P applications between 0 and 600 Kg P/ha. At 60 and 90 days after planting, corn was harvested. Significant increases in total N-yield and dry matter were observed as a response to P applications. For both harvest periods, corn grown under high P levels had significantly higher atom percent ^{15}N than corn grown under low P levels. The concentration of N in the shot indicated that corn grown under low P conditions was relatively less stressed for N compared to corn grown at high P levels. It is most likely that N-uptake curves for

corn grown under low P and high P are different and that this would result in different atom percent ^{15}N for corn grown at the different P levels.

Corn plants grown at different densities under identical soil conditions appear to be N-yield independent for atom percent ^{15}N. However, phosphorus stress which affects yield and N accumulation does affect the atom percent ^{15}N of corn. Results indicate that although a species may serve as a suitable reference plant for measuring N_2-fixation by isotope dilution in some conditions, the response of the reference plant to soil stress (such as phosphorus) must be considered. The suitability of a reference species for a given legume then becomes site specific.

7 Mechanisms controlling competition for nodule sites and nodule development in the soybean symbiosis.

Much effort has been directed toward finding strains of Rhizobium that are competitive with indigenous soil strains. This approach assumes that increasing the frequency of nodule formation by selected inoculum strains will enhance N_2 fixation in soils with native rhizobial populations. Most effort to identify competitive strains has involved inoculation with a marked strain and subsequent identification of nodules formed by the inoculum. Little attention has been paid to understanding the mechanisms that confer competitiveness to a strain or to understanding how the host controls infection by rhizobia and subsequent nodule development. Understanding these mechanisms will provide a basis for selecting host-strain combinations that are most likely to establish effective nodules in a soil with competing native strains.

We conducted a series of experiments to examine: 1) how strains and cultivars of soybean differ in the early stages of nodule formation and the effect of inoculation on root development; 2) whether strain virulence may be one mechanism which confers competitiveness on a strain; 3) how plant ontogeny affects the location of nodules on the root system of soybean; 4) whether reduction of N_2 controls the partitioning of photosynthate to nodules of soybean.

Results indicate that strains of Rhizobium japonicum and cultivars of soybean differ in the time required to form nodule primordia. Strains with normal competitive ability required 5 days from root emergence to exhibit nodule primordia on the tap root. Less competitive strains were delayed and formed few tap root nodules. A cultivar identified elsewhere as a "supernodulator" had root growth rates 50% greater than a normal cultivar at 5 days from planting. This same cultivar exhibited tap root nodulation

a greater distance down the tap root than the normal cultivar and continued the development of new tap root nodules for a longer period than a normal cultivar.

Early root development of all cultivars was inhibited by inoculation; the effects being relatively more prounounced and earlier in the "supernodulating" cultivar compared to the normal cultivar. Strains which were slow to develop nodule primordia also inhibited both tap and lateral root development compared to uninoculated controls. Within two days of appearance of the first nodule primordia (4 days supernodulator, 5 days competitive strains with normal cultivar, 6 days non-competitive strains with normal cultivar), the distance down the tap root where new nodule primordia appeared ceased to increase for all treatments except the "supernodulator." Results suggest that only virulent strains are abie to nodulate tap roots and this may be one mechanism which confers competitiveness on a strain.

In another experiment, we developed a vertical split-root system which enabled us to isolate sections of the growing root for inoculation. We employed a time course study (time points from planting to 9 days after planting) by applying inoculum to either the top alone, bottom alone, or top and bottom halves of the root systems. Total numbers of tap root nodules remained constant when plants were inoculated at germination (T-0), and 7, 22, and 47 hours later. However, even delaying inoculation by 7 hours caused almost a three-fold increase in the number of nodules on lateral roots. The proportion of nodules formed by the tap root declined to less than half with a 7-hour delay in inoculation. Total nodule number increased as inoculation was delayed and this remained true whether the top, bottom, or both root halves were inoculated. Total nodule number was constant for each time point regardless of whether half or the whole root system was inoculated.

Results suggest that tap root nodulation remains significant for a short period only and this is probably due to competition from an increasing number of available nodule sites on expanding lateral roots. These results also indicate that to a large degree, nodule occupancy by an inoculum strain placed at or near the emerging radicle is probably determined within one day of root emergence. If all available early nodule sites are not occupied by the inoculum strain, there would be little hope of maintaining a high proportion of nodules formed by introduced strains as rapidly expanding lateral roots explore soil containing native rhizobia.

After infection begins, the host must partition photosynthate to nodules for their development and functioning. Soybeans growing in soils with populations of rhizobia are

usually nodulated by strains with a range of effectiveness. In a sense, competition between strains for nodule sites in the infection process may extend to competition for plant photosynthate which then may condition development and function of nodules. We conducted an experiment to examine whether the host selectively partitions photosynthate to nodules that are reducing N_2 compared to inactive nodules. We nodulated both halves of a soybean split-root system with Rhizobium japonicum strain USDA 110. After 21 days we made the half root systems gas tight and flowed either air or a mixture of 80% argon and 20% oxygen by the half root system for 10 days. Treatments were 1) air on both sides; 2) argon and oxygen on both sides; 3) air on one side with the argon/oxygen mixture on the other. Using this technique, we were able to examine how N_2-fixation affects carbon partitioning to nodules formed by a genetically identical strain of Rhizobium. Plants were labeled with C^{14} prior to harvest followed by a 90 minute chase period. Respiration of the root systems and radioactivity of nodules, roots, and respired CO_2 was measured.

Results indicated that localized N_2-fixation stimulates both nodule and root development at the site of fixation. Current photosynthate was partitioned selectively to fixing nodules and their surrounding roots. Both total and specific radioactivity in roots and nodules was higher where fixation was taking place. This indicates that not only was the size of the sink created by development of fixing nodules larger than non-fixing nodules, but the intensity of the sink was also greater. The host selectively channels photosynthate to fixing nodules and their surrounding roots at a faster rate per unit weight of tissue than non-fixing systems. This mechanism helps explain how the host responds to nodulation by a mixture of strains from a rhizobial population that has a range of effectiveness for N_2-fixation. Results suggest that the nodule sink for photosynthate is no different than that of roots. Both sinks are enhanced by N assimilation; nitrate and ammonium enhance root growth and reduction of N_2 by nodules increases nodule development.

8 The interaction between environment, physiological development, and N_2 fixation by soybean from four maturity groups.

Five cultivars of soybean ranging from maturity group 00 to VIII were grown at elevations of 320 m, 660 m, and 1080 m. Soil and air temperatures differed by 5 degrees C between the extreme elevations. Non-nodulating isolines of each cultivar were included in the trial. Average seed yields of fixing cultivars generally decreased with increasing elevation: 4056 Kg ha^{-1} at 320 m, 3343 Kg ha^{-1} at 660 m, and 1647 Kg ha^{-1} at 1080 m. Lower

temperatures delayed the maturity of all cultivars by increasing the length of the vegetative stage (planting to RI) and early pod development (RIV). The duration of seed development was not affected by temperature. There was a strong interaction between site and cultivar for the length of vegetative growth period for non-fixing isolines. Longer maturing cultivars had extended vegetative periods compared to their fixing counterparts. Non-fixing cultivars had shorter seed filling periods than fixing cultivars at all elevations; early maturity groups reached physiological maturity faster than later groups. Data from non-fixing lines and in vitro mineralization studies of the three soils strongly suggest that N availability at the three sites was determined by both soil temperature and the mineralization potential of the soil and that soil N availability affected the rate of maturity of non-fixing cultivars.

The proportion of N derived from fixation ranged from less than 40% to more than 90% depending on site. Results indicated that under constant photoperiod soybean development is affected by temperature mainly by increasing the length of vegetative growth. Nitrogen fixation data suggest that greater conservation of soil N may be possible at high elevations in the tropics where soil N mineralization is reduced by low soil temperatures.

Yield data indicates that faster maturing varieties are more affected by elevation than longer maturity groups. Yield per unit time for the different maturity groups was the same at low elevation, but fast maturing varieties yielded substantially less per day at high elevation. Results suggest that fast maturing varieties may play an important role in sequential cropping systems at low elevations but not at high elevations.

9 The interaction between soil fertility, inoculation rate, and N_2 fixation by soybean.

A field experiment was conducted in a high P-fixing soil (Haiku clay, Tropohumult) to study the effects of strain selection for P tolerance, P fertility, and inoculation rate on nitrogen fixation by soybean. A 2x3x3 split-split plot design was employed with P rate (100 and 600 Kg P ha^{-1}), strain of Rhizobium japonicum (USDA 110 and 142 tolerant and intolerant, respectively, to low P in vitro applied in either single or mixed inoculation) and inoculation rate (10^2, 10^4, 10^2 cells $seed^{-1}$) as the main plots, subplots, and sub-subplots, respectively. Rhizosphere counts and nodule identification indicated no difference between strains in ability to colonize the rhizosphere or form nodules at low P. Yields were greatly reduced by low P fertility. Yields were increased with increasing inoculation rates and this increase was enhanced at high P compared to low P. Results indicate that selec-

tion of Rhizobium japonicum for tolerance to low P will probably not be a fruitful approach to increasing nodulation and N_2-fixation in soils with low P availability. This experiment demonstrates a synergism between two management inputs: inoculation and P-fertilization.

A corollary growth room investigation was conducted with the same two strains of Rhizobium japonicum to examine whether plant or microbial sensitivity first limited nodulation of soybean under P-stressed conditions. We evaluated the effect of soil P on rhizosphere growth, nodule formation, and competition for nodule sites by Rhizobium japonicum (USDA 110, USDA 142, tolerant and intolerant, respectively, to low P). Two densities of inoculation (6.0 x 10^2 cells g^{-1} soil, 1.3 x 10^1 cells g^{-1} soil) for both strains were imposed on half of a soybean root system growing in P-fixing soil supplied with either 0, 50, or 600 mg P Kg^{-1} soil (P_0, P_{50}, P_{600}). The other half of the root system was supplied with 1500 mg P Kg-1 soil. A control (P_0 on both sides) was also employed. The high inoculation density treatment produced three times more nodules than low density. Adding P increased nodule number by 25% in the low inoculum density treatment for both strains; there was no response to P at high density. The control had less than half the nodule number and shoot N compared to plants inoculated on the P_0 side with P 1500 on the other side. Nodule occupancy of USDA 142 was 64% and 66% at P_0 and P_{600}, respectively, when the two strains were mixed. USDA 142 grew more than USDA 110 in the rhizosphere reegardless of P level. Host P nutrition affects nodule formation in low P soil more than rhizobial tolerance to low P. Tolerance of a strain to low P in vitro did not enhance competitive ability for nodule formation at P_0 nor growth in the rhizosphere.

Results indicate that although extremely low P-availability at the root surface can affect nodulation, the overall P status of the shoot has a greater affect on nodulation. Efforts to increase N_2-fixation in P-stress should be directed toward breeding for plant tolerance to low P.

BNF in Winged Beans Introduced to the Pacific Islands

The original goals of the project were nearly met in 1985. Winged beans had been previously surveyed in Guam to determine the extent of nodulation and nodules were retrieved as sources of Rhizobium isolates. The most effective isolates were determined through greenhouse trials. Four serologically distinct strains were subsequently selected for a field trial on Guam in 1985. These strains--TAL 169, TAL 651, TAL 1425, and TAL 1672--were incorporated into a peat-based ioculant. Fluorescent

antibodies for each strain were produced. The results of the field experiment showed a response to inoculation at both maximum and farm fertility levels. Also, higher seed yields were obtained with inoculation over both fertilizer N and the control treatments. Serotyping of the nodules from this experiment is underway.

Also in 1985, tissue culture techniques were studied for examining stress tolerance in winged beans under cold conditions. Callous production was initiated and is currently maintained for two winged bean cultivars. Different tissue culture media were employed in an effort to stimulate root, shoot, and whole plant production.

Mutagenesis methodologies were used as a tool to screen for three plant types. 1) Non-nodulating lines of winged bean mutants for use in fundamental studies of nitrogen fixation in this species. Non-noduating plants can be used as a means of assessment of the level of nitrogen fixation. By using non-nodulating lines in field conditions where there are large numbers of effective Rhizobium already established, a comparison can be made between the non-noduating and nodulating plants to determine the effect of N-fixation on yield. 2) Winged bean variants which nodulate in the presence of high nitrate concentrations and which are useful in the study of the regulation of nodule development. 3) Erectoid plants which do not require a trellis support system for growth. One of the factors inhibiting large-scale production of winged beans is the climbing habit of this crop. Selection of self-standing or dwarf varieties would alleviate this problem. The variety "Lunita" was treated with a mutagen, ethyl methanesulfonate, and approximately 500 plants were planted in the field for subsequent collection of seed.

PRC Soybean Study

Under sponsorship of the U.S. National Academy of Sciences Committee on Scholarly Communication with the People's Republic of China, a NifTAL graduate student initiated studies on soybean rhizobia from their center of origin, China. Field and laboratory experiments examined the interactive effects of cropping systems, soil type, and inoculation on the competitiveness and persistence of introduced and indigenous Rhizobium japonicum.

Populations of soybean rhizobia were dominated by fast-growing and acid producing strains (R. fredii) in soil that was previously cropped with soybean. The population in soil that had long been used for rice production was predominately composed of typical slow-growing Rhizobium japonicum.

Competition studies with isolates showed the fast-growing strains to be extremely competitive, however, when

both types were introduced into a soil free of Rhizobium japonicum, the slow-growing strains formed most of the nodules on soybean. Cropping system (sequential rice-rice, rice-soybean, or soybean-soybean) did not affect the Rhizobium population sufficiently such that inoculation increased soybean yields after two rice crops.

Inoculant strains did form significantly more nodules on soybean following rice than on soybean following soybean. This suggests that the numbers of rhizobia in the indigenous population following rice was less than following soybean.

IMPROVEMENT OF LEGUME SYMBIOSES IN INFERTILE SOILS

The University of California-Davis is subcontracted under the direction of Don Munns to conduct research in the area of soil stress tolerance. The objective of the project goal is to lower the cost of soil management, improve crop performance, and guide the choice of legumes for cropping systems by defining their tolerances to components of soil infertility.

Research during 1985 centered on experiments in two areas. First, the nutrition of rhizobia was researched through experiments on the comparative impact of different soil factors and rhizobia and organic phosphate. Second, salinity was characterized on three major legume species--Cicer arietinum, Leucaena leucocephala, and Phaseolus lunatus. Following are research highlights from this year's work.

NUTRITION OF RHIZOBIA

Comparative Impact of Different Soil Factors

Most studies of environemntal stresses on rhizobial growth have characterized isolated single factor effects. We have completed a comparative study of effects and interactions of several limiting factors: carbon (sugar), nitrogen sources, phosphate, calcium, acidity, and Aluminium toxicity. Each factor was tested with Bradyrhizobium strains USDA 100 and 142, at two levels. The lower level corresponded to conditions in an infertile soil solution, the upper level to conditions in typical laboratory media. Deficiencies of Ca, N, P, and carbon at high pH (5 to 7) did not reduce growth greatly when compared with the toxic effects of Al and H. However, significant responses to P and Ca developed when these stresses were imposed in combination with low pH or high Al, combinations likely to occur in acid soils.

Rhizobia and Organic Phosphate

In soils that supply orthophosphate slowly, less-available forms of P become relatively more important. Notable is phytic acid, which accumulates in substantial quantity adsorbed to clays or precipitated with Fe and Al. Strains of Bradyrhizobium differed in their ability to attain large populations in liquid media with phytic acid as the primary source, but the nature of the strain difference is still not clearcut. Inorganic phosphate impurities are difficult to eliminate from phytic acid solutions, and we have found that phytic acid is less stable than usually assumed. Our current working hypothesis is that strain differences in apparent ability to use phytate is less stable than usually assumed.

Our current working hypothesis is that strain differences in apparent ability to use phytate reflect efficiencies in using the orthophosphate present as impurity rather than inherent differences in ability to hydrolize phytic acid. We have developed a simple method for separating orthophosphate from phytic acid and used it to study the stability of sodium phytate at different temperatures (5 to 54 C) and pH (4.4 to 10.0) under aseptic conditions. Phytic acid released orthophosphate at nutritionally significant rates, especially at the lowest pH and above 30 C - a temperature likely to occur in upper soil horizons where phytate is most abundant.

Salinity and Legumes

We have characterized salinity effects on three more major legume species: Cicer arietinum, Leucaena leucocephala, and Phaseolus lunatus. In none of these is there evidence of important salinity effects operating specifically and directly on rhizobia, nodulation, or nodule function. Cicer arietinum is highly salt sensitive (30 mM NaCl gives 50% growth reduction), and 170 cultivars showed no useful variation in tolerance. Survival of high salt levels (100 mM) did not predict a cultivar's ability to grow well at less severe levels. Salt damage arises primarily from Na accumulation in older leaves, greatly aggravated by low humidity. The plant has poor control of transpiration or of flux of Na and Cl to the shoot. Leaf Na is a useful diagnostic indicator of both stress and sensitivity. Two papers have been submitted (Australian Journal Plant Physiology) and two are in press (Plant and Soil and Journal of Experimental Botany).

Leucaena's salt tolerance depends heavily on Ca supply. At low Ca (0.5 mM in solution culture), it is little more tolerant than chickpea, but at higher Ca (5 mM) 100 mM NaCl is required to produce significant growth reduction. Two papers have been submitted (Plant and Soil).

Phaseolus lunatus is evidently a very tolerant grain legume. Growth of a commercial cultivar is reduced significantly at 200 mM but not at 100 mM NaCl. The plants effectively excluded Na from the shoot system. We are seed-increasing some old Navajo selections that might be more salt tolerant.

ECONOMIC EVALUATION OF BNF

The major activity of the University of Minnesota (UM) subcontracted agricultural economics of BNF component of the NifTAL Project during calendar year 1985 was field work in Thailand carried out by the graduate research assistant assigned to this project, Mr. Keith Fuglie. Additional funding for field work and travel to and from Thailand was also supported in part by a separate grant from AID/S&T/AGR.

Work in Thailand was carried out between October, 1984, and September, 1985. Fuglie's visa for the year in Thailand was obtained by the Thailand National Research Council (NRC) with status as a visiting researcher and his sponsor to the NRC was Khon Kaen University (KKU). He was appointed as a visiting researcher in the Farming Systems Research Program (FSRP) of the Faculty of Agriculture, KKU. All of the field work and other analyses carrried out were done as a part of the FSRP. Providing an essential source of intellectual and infrastructural support and advisory assistance we Dr. Terd, FSRP leader, and Dr. Doug Beck, former NifTAL Thailand BNFRC Director.

The major current thrust of the KKU-FSRP that relates to BNF is in the use of legumes--primarily peanuts--in rice-based rotations. The overall FSRP program is on developing and testing rice-based cropping systems in rainfed areas of Northeast Thailand. Several of the cropping systems under study contain legumes grown in rotation with rice or field crops. Currently, the most attention is being placed on rice-peanut rotations, usually grown as peanuts-after-rice, although innovative farmers have been found who are practicing a peanuts-before-rice system with apparent success. There are also substantial research efforts at KKU and at the Department of Agriculture, Ministry of Agriculture (DOA), in Rhizobium strain selection and legume cultivar (mainly peanuts and soy beans) selection to increase nitrogen fixation.

The UM BNF Economics subcontract activities during the year of field work in Thailand can be classified in four categories: 1) conducting economic analysis of BNF data being collected by other researchers on independent projects; 2) participating in FSRP multi-disciplinary field research through the addition of an economics component; 3)

providing leadership for additional FSRP field studies whose primary focus is BNF economics; and 4) conducting some background studies in regional agricultural development-issues and the analyses of the secondary data from general statistical sources as well as from project records obtained from various government agencies.

In category 1, collaboration with Khon Kaen University and DOA researchers on both past and on-going research projects was done to measure the economic benefits derived from increased nitrogen fixation. The first such study was conducted by Dr. Nantakorn Boonkerd (DOA). It was a multi-location test of response to inoculation of several soybean cultivars with Rhizobium at several levels of nitrogen fertilization. The second study is collaborative with Dr. Rennie (IDRC-Canada) and Dr. Nantakorn. This is a newly initiated study, using isotope tracing to measure nitrogen fixation by several popular cultivars of soybeans using several different Rhizobium strains. The third study, being conducted by Mr. Banyong, Microbiologist at KKU, is one which attempts to measure nitrogen fixation by peanuts.

In the category 2 studies, the KKU-FSRP Team collaborated with the Department of Agricultural Extension, Ministry of Agriculture (DOAE) in a multi-locational field test in Khon Kaen Province of a peanuts-after-rice crop rotation. The economic component was to measure the economic viability of that crop rotation in comparison with other farm and household activities competing for the same farm resources.

In the category 3 studies--involving primary data collection--an on-going project was established within KKU-FSRP to measure the rotation effect of adding peanuts-after-rice to a rice-only cropping system in Khon Kaen Province. Co-leaders of this project are Dr. Viriya and Mrs. Nongluck, of the KKU-FSRP Team, who are providing continuing and on-going leadership of this activity. The farms selected for this study are a sub-sample of the farms involved with the cropping system field test mentioned in category two. A second study in this category is of a peanuts-before-rice crop rotation being practiced in some areas of Korat Province. Here farmers are using short maturity peanuts planted at the beginning of the rainy season in upper paddy fields. The peanut harvest is followed by rice transplanting, if rain is sufficient.

An example of category 4 includes a historical study of rice yield trends in northeastern Thailand. Another example is an economic analysis of nitrogen fertilization studies carried out in several rainfed rice growing areas of the Northeast Region.

Mr. Fuglie's activities after returning to the University of Minnesota in September, 1985, through the remainder

of the year, were completing several econometric analyses of fertilizer data which were started in Thailand but for which larger computer capacity was needed, and preparing first drafts of several papers. A list of the tiles of papers which are now in their first draft stage and titles of seminars presented as part of the subcontract are listed below. A condensation of part of the fertilizer paper has been submitted as a contributed paper to the 1986 annual meetings of the American Agriculture Economics Association.

Papers in draft form: 1. The economic viability of peanuts-after-rice cropping system in Khon Kaen Province; 2. Trends in rice production and yield in Northeast Thailand, 1947-1984; 3. Varatal choice and nitrogen fertilization of rice in rainfed areas of Northest Thailand. Future Papers: 4. Economic benefits from soybean inoculation in Northeast Thailand; 5. The rotation affects of peanuts on rice yields in peanuts-after-rice cropping system in Khon Kaen Province; 6. The economic viability of peanuts-before-rice cropping system in Korat Province; and Seminars: Paper #1 was presented at a meeting of regional DOAE leaders at Khon Kaen, 1 September 1985.

USDA COMPETITIVE GRANT

Host Control of Nodule Formation and Development in Soybean/*Rhizobium* Nitrogen Fixing Symbiosis

Research under this grant examines the role of the leguminous host *Glycine max* in regulating the process of infection and nodulation by its microsynbiont, *Bradyrhizobium japonicum*. The split-root growth system is being used as a research tool which allows the partitioning of host and microsymbiont events during the infection and noduation process. Of specific interest is the phenomenon of host supression of nodulation on half of its root system when the other half has been previously exposed to *Rhizobium*. Eight soybean cultivars were screened in the split-root growth system to examine this suppression effect. Results indicate that relative root growth and number of infection sites available to the primary inoculum is a critical factor in determining the degree of later nodule suppression on the secondary side. Results of experiments using *Bradyrhizobium* strains of differing N-fixing capabilities imply that the effectiveness of the primary inoculum and host compensatory mechanisms to meet growth potential also affect the suppression of subsequent nodule development.

ABSTRACTS OF 1985 PUBLISHED PAPERS

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

Effects of calcium at 300 and 1500 M on P nutrition were assessed in eight strains of *Rhizobium meliloti* in defined liquid medium. Evaluations included: P storage from "luxury" external concentration (1000 M P); utilization of stored P after transfer to unreplenished low-P medium (0.06 M); and growth at low concentrations of P buffered at 5, 0.5, and 0.06 M with an iron oxide dialysis system. The strains stored P in luxury medium, but unlike other rhizobia, they needed high Ca to utilize the stored P. They either grew or died following transfer to low-P medium, depending on the Ca concentration and the Ca concentration at which they had grown previously. Ability to grow in media buffered at low P concentrations also contrasted with that of other rhizobia, in two respects: no strain of *R. meliloti* grew at 0.06 M P, regardless of Ca concentration; and some strains needed high Ca to grow at 0.5 and 5 M P. Two isolates from *Medicago rugosa* and *Melilotus indica* were less Ca-demanding than six isolates from *Medicago sativa*. Previous reports that *R. meliloti* has low calcium requirements may be correct only for the luxury P levels that are conventional in defined media. Our evidence for high Ca requirement at realistic P concentrations agrees with data from soil experiments.

Rhizobium Relationships. Burton, J.C. In. Clover Science and Technology, Agronomy Monograph #25, 161-184.

The clovers, like many other legumes, were cultured and valued for their soil-building properties for centuries before the magic of the legume nodule was known. The cause or significance of the nodules on the roots of leguminous plants was not understood until Hellriegel and Wilfarth (1888) reported on their classical experiment in which they showed that nodules on the pea roots were the source of nitrogen for the plant's growth.

Many clover failures undoubtedly occurred from lack of effective nodule bacteria during this early period, particularly where clover was planted for the first time; but fortunately, the failures did not prove to be a severe deterrence to the growing popularity of this legume. It was a common practice when seeding new fields to transfer soil from a field that had previously grown a successful crop of the same clover. Although the reasons for doing this were not understood, the nodule bacteria were being supplied in the old soil.

Laboratory-grown cultures of the nodule bacteria were produced shortly after Beijerinck (1888) isolated rhizobia from nodules, but the early results from these laboratory-grown cultures were very disappointing. The soil transfer method remained more dependable than use of laboratory-grown cultures for many years (Fred, et al., 1932). In light of our present knowledge of the delicate relationship between the nodule bacteria and their host, and the references of each symbiont, this result should not be surprising.

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

Described is a system of worldwide trials for testing response to legume inoculation, the International Network of Legume Inoculation Trials (INLIT). The basic INLIT experiment is described in detail: formulation of the research design, the coordination and servicing of trials, and the analysis of data on a single and multiple location basis. Included is a treatment of the data analysis of field experiments containing nonorthogonal comparisons. Secondary benefits of the trials have been identifying research workers and policy makers in developing countries, and organizing these persons into an organization for expediting research and information flow. Acceptance of the concept by many legume inoculation workers has indicated the validity of the concept, and feedback from cooperators has indicated that the INLIT program has been successful.

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

Soybean rhizobia were isolated from two soils with different cropping histories from Hubei Province in central China. The first, from Honghu County, has been under soybean cultivation for decades. All of the isolates obtained from nodules on soybeans growing in this soil were fast-growing, acid-producing rhizobia. However, slow-growing, alkali-producing isolates were obtained at higher dilutions of the same soil. The second soil, from Wuchang County, has been under rice cultivation with no record of previous soybean cultivation. All of the soybean rhizobia recovered from this soil, and at higher dilutions of the soil, were typical slow-growing, alkali-producing isolates. The isolates from both soils were grouped by using intrinsic antibiotic resistance, gel immunodiffusion, and fluorescent-antibody procedures. Representative isolates were tested for symbiotic effectiveness with four soybean cultivars (Peking, Davis, Williams, and Ai Jiao Zao) in a pot experiment. There were significant cultivar-rhizobial interactions.

Moreover, on each cultivar, there was at least one fast-growing isolate among these new rhizobia that was as effective as the highly effective slow-growing reference strain USDA 110.

Selective Medium for Recovering Specific Populations of Rhizobia Introduced into Tropical Soils. Habte, M. Appl Env Microbiol 50(6): 1553-1555.

Experiments were designed to evaluate the usefulness of antifungal agents and streptomycin for recovering low densities of rhizobia inoculated into tropical soils. The results showed that yeast-mannitol agar (pH 6.0) containing 50 g of streptomycin, 400 g of cycloheximide and 50 g of benomyl or chlorothalonil per ml was the best selective medium.

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

Rhizobium are the bacteria capable of forming a nitrogen-fixing symbiosis with leguminous plants. Traditionally, their classification has been based on specific host infectivity with the recognition of two broad groups of fast-and slow-growing strains (Jordan and Allen, 1974). In addition to growth rates, the two groups are distinguished by several important differences, which has led to the proposed establishment of a separate genus for slow growers (Bradyrhizobium gen. nov.), while retaining fast growers in the genus Rhizobium (Jordan, 1982).

The soybean-nodulating bacteria, R. japonicum, have been classified as slow growers. Recently, however, Keyser et al. (1982) reported the existence of fast-growing isolates obtained from soil and nodules collected in China. Examination of the 11 fast-growing isolates for microbiological, genetic, and symbiotic properties has shown them to be distinct from the slow growers. They represent a unique and valuable addition of Rhizobium germplasm. In this article we present an overview of microbiological and symbiotic properties of the fast-growing soybean rhizobia. Results from genetic studies are presented elsewhere in this volume by Atherly et al. Several investigators are currently examining these fast growers for various attributes, and knowledge of them should increase rapidly.

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

The effect of several biotic and abiotic factors on the pattern of competition between two strains of Rhizobium japonicum was examined. In two Minnesota soils, Waseca and Waukegan, strain USDA 123 occupied 69% (Waseca) and 24% (Waukegan) of the root nodules on Glycine max L. Merrill cv. Chippewa. USDA 110 occupied 2% of the root nodules in the Waseca soil and 12% of the nodules in the Waukegan soil. Under a variety of other growth conditions--vermiculite, vermiculite amended with Waseca soil, and two Hawaiian soils devoid of naturalized Rhizobium japonicum strains--USDA 110 was more competitive than USDA 123. The addition of nitrate to or the presence of antibiotic-producing actinomycetes in the rhizosphere of soybeans did not affect the pattern of competition between the two strains. However, preexposure of young seedlings to USDA 110 and USDA 123 before transplantation into soil altered the pattern of competition between the two strains significantly. In the Waseca soil, preexposure of cv. Chippewa to USDA 110 for 72 h increased the percentage of nodules occupied by USDA 110 from 2 to 55%. Similarly, in the Hawaiian soil Waimea, nodule occupancy by USDA 123 increased from 7 to 33% after a 72-h preexposure.

Annual, Seasonal and Diel Variation in Nitrogen Fixing Activity by Inga jinicuil, a Tropical Leguminous Tree. Roskoski, J.P. and C. van Kessel. Oikos 44: 306-312.

Patterns in nitrogen-fixing activity by Inga jinicuil Schl., a leguminous shade tree in Mexican coffee plantations, were monitored over a three-and-half year period using acetylene reduction.

Year to year variation was unexpectedly large; mean annual fixation equalled 35 kg N ha^{-1} yr^{-1}, which constitutes a significant nitrogen input to the coffee ecosystem. Nitrogen-fixing activity occurred throughout the year but was highest during the summer and autumn when precipitation and temperature were at a maximum and when the majority of tree growth and reproduction occurred. I. jinicuil flowered twice annually and nodular activity peaked once during each reproductive cycle, with maximum activity after flowering in the first reproductive cycle and before flowering in the second.

Diel fluctuations in nitrogen fixation rates were obtained on most but not all sampling dates, but the observed patterns of activity varied from date to date. Aside from an activity peak that occurred at 1900 hours, averaged rates of nodular activity were remarkably constant throughout the day. Nodules from seedlings fixed 35% more

nitrogen than 30-yr-old-trees but had a similar diel activity pattern.

Overall, the results show that variability in nitrogen-fixing activity was large between years, pronounced but explainable between months, and relatively small between hours of the day. The timing of maximum and minimum activity, both seasonally and daily, differed significantly from what has been reported for most other nitrogen-fixing species.

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

The Rhizobium leguminosarum host-range (Sym) plasmid, pJB5JI, is a 130 Mdal conjugative plasmid which carries the genes for host-specifity (i.e., for peas) and some of the genes for nitrogen-fixation. We have transferred plasmid pJB5JI into different species of Rhizobium and examined the symbiotic properties of the resulting transconjugants. The donor strain R. leguminosarum 6015 (pJB5JI), was only moderately effective on peas. Transconjugants of a Nod^- mutant of R. trifolii, #2, became highly effective on peas, but did not regain their ability to nodulate clover. Transconjugants of a Nod^+ strain of R. trifolii, 0403, could nodulate peas, but only ineffectively, and retained the ability to nodulate clover effectively. All reisolates of #2 transconjugants from pea nodules had gained the ability to form ineffective nodules on clover. Reisolates of 0403 transconjugants from both clover and pea nodules exhibited three different symbiotic phenotypes: They could nodulate (1) both peas and clover effectively; (2) clover effectively but peas ineffectively; or (3) only clover effectively. Trnasconjugants made with a fast-growing soybean-Rhizobium were unable to nodulate peas, but retained the ability to nodulate soybeans. The presence of the plasmid in these transconjugants was ascertained by back-crossing to a Nod^- of R. leguminosarum. In all the studies, the identity of the donors, the recipients and the transconjugants were confirmed by their antibiotic and auxotrophic markers, as well as by immunofluorescence and immunodiffusion using strain specific antisera. In this study, we have shown that plasmid pJB5JI is readily transferable to different species of Rhizobium. However, its symbiotic expression varies depending on the genetic background it resides in.

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

We examined the relationship between P nutrition of soybean [Glycine max (L.) Merr. cv. 'Davis'] and N_2 fixation by five strains of Rhizobium japonicum ranging from an

ineffective (SM-5) to a highly effective (USDA 110) symbiosis with soybean. Phosphorus at 0, 50, 125, and 400 mg P kg^{-1} soil (P_0, P_{50}, P_{125}, P_{400}) as $Ca(H_2PO_4)_2 \cdot H_2O$ was applied to pots containing 2.7 kg of a P-fixing humoxic tropohumult free of R. japonicum. Treatments consisting of +N and five strains of R. japonicum and the four P treatments were arranged in a complete factorial replicated three times. With 400 mg P kg^{-1} soil treatments ranked +N > USDA 110 > USDA 31 > USDA 123 > USDA 33 > SM-5 for shoot dry weight and accumulation of N in the shoot. There were no significant strain effects for shoot dry weight, shoot N or nodule activity at P . Strain rankings were the same at the intermediate levels of P but differences were smaller. Although nodule number was somewhat enhanced by P, it bore no relationship to strain effectiveness and was not a factor limiting N_2 fixation at low P. Nodule dry weight and nitrogenase activities significantly increased with P additions. The concentrations of N and P in the shoot also increased with P additions. The concentrations of shoot P and N were higher in plants provided mineral N at the low and intermediate P levels than in the inoculated plants. The results show that P nutrition is important in interpreting the N_2 fixation capability of strains of Rhizobium. Synergism between P and Rhizobium inputs requires that to obtain maximal response to the application of one input necessitates employing the other at maximum levels.

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

Fully grown broth cultures of various fast- and slow-growing rhizobia were deliberately diluted with various diluents before their aseptic incorporation into autoclaved peat in polypropylene bags (aseptic method) or mixed with the peat autoclaved in trays (tray method). In a factorial experiment with the aseptic method, autoclaved and irradiated peat samples from five countries were used to prepare inoculants with water-diluted cultures of three Rhizobium spp. When distilled water was used as the diluent, the multiplication and survival of rhizobia in the peat was similar to that with diluents having a high nutrient status when the aseptic method was used. In the factorial experiment, the mean viable counts per gram of inoculant were log 9.23 (strain TAL 102) > log 8.92 (strain TAL 82) > log 7.89 (strain TAL 182) after 24 weeks of storage at 28 degrees C. The peat from Argentina was the most superior for the three Rhizobium spp., with a mean viable count of log 9.0 per g at the end of the storage period. The quality of inoculants produced with diluted cultures was significantly (P=0.05) better with irradiated than with autoclaved peat, as shown from the factorial

experiment. With the tray method, rhizobia in cultures diluted 1,000-fold or less multipled and stored satisfactorily in the presence of postinoculation contaminants, as determined by plate counts, membrane filter immunofluorescence, and plant infection procedures. All strains of rhizobia used in both the methods showed various degrees of population decline in the inoculants when stored at 28 degrees C. Fast- and slow-growing rhizobia in matured inoculants produced by the two methods showed significant ($P < 0.01$) decline in viability when stored at 4 degrees C, whereas the viability of some strains increased significantly ($P < 0.01$) at the same temperature. The palnt effectiveness of inoculants produced with diluted cultures and autoclaved peat did not differ significantly from that of inoculants produced with undiluted cultures and gamma-irradiated peat.

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

Using a split-root technique, roots of soybean plants were divided between two pots. In one of the two pots, two maize plants were grown and half of those pots were inoculated with the vesicular arbuscular mycorrhizal (VAM) fungus, Glomus fasciculatus. Fifty-two days after planting, ^{15}N-labeled ammonium sulfate was applied to the pots which contained only soybean roots. Forty-eight hours after application, significantly higher values for atom per cent ^{15}N excess were found in roots and leaves of VAM-infected maize plants as compared with the non-VAM-infected maize plants. Results indicated that VAM fungi did enhance N transfer from one plant to another.

SECTION III: APPENDICES

APPENDIX I

INLIT COOPERATOR'S COUNTRIES

ARGENTINA
BANGLADESH
BRAZIL
BURMA
CAMEROON
COLOMBIA
DOMINICAN REPUBLIC
ECUADOR
EGYPT
ETHIOPIA
FIJI
GHANA
GUATEMALA
HAUTE VOLTA
HONG KONG
INDIA
INDONESIA
JAMAICA
KENYA
MALAYSIA
MAURITIUS
MEXICO
NEPAL
NIGER
NIGERIA
PAKISTAN
PRC
PERU
PHILIPPINES
PUERTO RICO
RWANDA
SAUDI ARABIA
SOUTH AFRICA
SRI LANKA
SUDAN
SWAZILAND
TAIWAN
TANZANIA
TURKEY
UGANDA
WEST INDIES
ZAMBIA

APPENDIX II

TABLE 1. Percent yield increase of soybean from inoculation with *Rhizobium japonicum* at four Zambian experimental sites under maximum inputs.*

	Bossier**	Hernon	Magoye	Santa Rosa	100 Kg N
Site	----------%----------				
Golden Valley	295	130	32	58	
Mufulira	81	92	232	94	
Nanga***	30	24	23	20	17
Msekera	22	27	26	60	42

* Includes application of Lime, 80 Kg P, 120 Kg K. Average yields of inoculated plants were (Kg seed ha^{-1}): Nanga 2484; Golden Valley 1873; Mufulira 1522; Msekera 1579. 100 Kg N data is averaged across all cultivars at the two sites.

** Bossier is a North American cultivar. The other three are Zambian cultivars which nodulate with some strains native to Zambian soils.

*** Nanga and Msekera data are the average of two experiments. One testing seven strains (the top four strains used in these results) the other testing management practices inoculating with USDA 110 only.

TABLE 2. Most probable number (MPN) counts of the number of invasive rhizobia in three fields at Mkushi

Host Plant	Virgin Soil	Soybean Field No Inoculation	Soybean Field With Inoculation
	---	Number of rhizobia g^{-1} soil	---
Mungbean	5.1×10^2	3.0×10^2	1.7×10^2
Hernon*	1.7×10^2	1.7×10^5	1.7×10^4
Kaleya	1.8×10^0	1.7×10^2	5.1×10^3

* Hernon expresses the character of nodulation with rhizobia native to Zambian soil. Kaleya is somewhat resistant to nodulation with these strains and resembles North American varieties for this character.

APPENDIX III

1985 CULTURE REQUESTS

Country	Number of Requests	Number of Cultures
Australia	3	48
Canada	3	67
Colombia	3	22
Republic Federal Islamique des Camores	1	3
Dominican Republic	1	2
Egypt	2	132
Finland	1	13
Ghana	1	4
Haiti	2	14
India	13	136
Indonesia	2	13
Kenya	2	22
Korea	2	2
Malaysia	2	61
Mexico	6	38
Morocco	1	2
New South Wales (Aust.)	1	7
Nigeria	2	14
Panama	2	18
Peru	3	11
Philippines	4	28
Saudi Arabia	1	6
Senegal	3	44
Sri Lanka	3	11
Sudan	3	21
Switzerland	1	3
Taiwan	1	3
Thailand	5	88
Tunisia	1	18
Turkey	1	103
USA	24	138
Uruguay	1	3
Venezuela	2	32
Zimbabwe	2	24
TOTALS 34	104	1,151

APPENDIX IV

Antisera Requests Filled

Country	Antisera for	Number of Requests
Brazil	TAL 102	1
	TAL 377	1
	TAL 379	1
Canada	TAL 102	1
	TAL 377	1
	TAL 480	1
	TAL 1148	1
	TAL 1379	1
	TAL 1397	1
	TAL 1400	1
	TAL 169	1
	TAL 22	1
	TAL 420	1
	TAL 441	1
	TAL 644	1
	GARG	1
Chile	TAL 1145	1
	TAL 582	1
	TAL 209	1
	TAL 173	1
	TAL 309	1
	TAL 82	1
Egypt	TAL 377	2
	TAL 102	2
	TAL 379	3
	TAL 638	2
	TAL 634	2
	TAL 640	2
	TAL 169	2
Haiti	TAL 169	1
	TAL 1000	1
	TAL 569	1
	TAL 1127	1
	TAL 182	1
	TAL 1376	1
India	TAL 569	2
	TAL 1145	2
Nigeria	TAL 582	2
	TAL 1145	2
Pakistan	TAL 169	2
	TAL 441	2
	TAL 420	2
	TAL 379	2
	TAL 102	2
	TAL 377	2
	GARG	1
Peru	TAL 360	1
	TAL 1373	1
	TAL 1372	1
Sri Lanka	TAL 1383	1
	TAL 582	1
	TAL 1145	1
South Africa	TAL 377	1
	TAL 379	1
	TAL 102	1
Taiwan	TAL 102	1
	TAL 377	1
	TAL 379	1
Thailand	GARG	1
Turkey	TAL 1376	1
USA	TAL 169	3
	TAL 173	4
	TAL 658	3
	TAL 102	4
	TAL 377	2
	TAL 379	2
	TAL 1376	2
	TAL 1383	5
	TAL 182	1
	TAL 82	1
	TAL 377	1
	GARG	2
Zambia	TAL 102	1
	TAL 377	1
	TAL 379	1

APPENDIX V

1985 PARTICIPANTS IN NifTAL TRAINING

Graduate Assistantships

Person	Country	Degree
Tafiqul Aziz	Bangladesh	Ph.D.
Steven Dowdle	USA	Ph.D. (completed)
Thomas George	India	Ph.D.
Wilson Kagabo	Liberia	M.S.
Naseer Mirza	Pakistan	M.S.
Ronnie Nyemba	Zambia	M.S.

Internships

Person	Country	Duration of Stay
Velittin Gurgun	Turkey	5 months
Muhrizal Sarwani	Indonesia	6 months
Rolando Torres	Philippines	5 months

CIAT/Porto Alegre MIRCEN/NifTAL Course (November-December)

Person	Institute/Country
Renato Valenzuela Bedregal	CIAT/Bolovia
Stela D. Vieira Midlej Silva	CEPLAC/CEPEC/DIFIT Brasil
Reinaldo Bertola Cantarutti	CEPEC/Brasil
Joaquin Garcia Pena	ICA/Colombia
Justo A. Barros Henriques	ICA/Colombia
German Hernandez Cordoba	CNP/Costa Rica
Lidieth Uribe Lorio	Centro Investigaciones Agronomicas/Costa Rica
Marcial E. Guzman Alburez	ICTA/Guatemala
Maria I. Castellanos Arrecha	ICAITI/Guatemala
Juan Francisco Aguirre Medina	INIFAP/Mexico
Anselmo Cigarroa de Aquino	INIFAP/Mexico
Esteban Arosemena Jaen	IDIAP/Panama
Margarita X. Cornejo Montenegro	University of Panama/Panama
Silos Gonzalez del Aguila	IVITA/Peru
Cesar Augusto Reys Atac	IVITA/Peru
Martin M. Mbewe	Ministry of Agriculture/ Zambia
Mirta Lopez Gutierrez	Institute of Animal Science/Cuba

APPENDIX VI

CIAT/PORTO ALEGRE MIRCEN/NifTAL COURSE LECTURES

	LECTURE TITLE	LECTURER	NUM OF HO
1	Objectives of the course (instrument) Demonstration of audiotutorial unit	D Harris	1
2	Introduction to legumes and legume-rhizobia symbiosis. Importance and benefit of N_2 fixation	J Kolling	1
3	Methods of enumerating rhizobia	H Hoben	2
4	N contribution of legumes in different systems (rotation green manure, N-transfer)	D Scholles	1
5	Importance of N_2 fixation in legume breeding and selection programs	J Kipe-Nolt	1
6	Establishment of the need for inoculation, evaluation of N fertilizer response, evaluation of nodulation, rhizobia screening and strain selection for forage and grain legumes.	R Bradley	2
7	Experiences in adapting BNF technology for use in the Cerrado of Brasil	R Peres	1
8	Methods of inoculation of seed and soil appropriate for grain and pasture legumes; survival of rhizobia in inoculant and on seeds	J Kolling	1
9	Limiting factors (plant, rhizobia, environment)	D Scholles	1
10	Nutritional factors affecting N_2 fixation	A Franco	1
11	Nineral N transformations in the soil	M Tedesco	1
12	Limiting fertility factors in tropical soils; importance of sample collection	D Harris	1
13	Taxonomy of rhizobia	J Jardim-Freire	1
14	Forage legume: importance, cultivation, nutrition, management, association with grasses	N Seiffert	1
15	Grain legume: importance, cultivation, nutrition, management, association w/other crops	J Costa	1
16	Extension and promotion of legume-inoculants	E Meurer	1

APPENDIX VI (continued)

CIAT/PORTO ALEGRE MIRCEN/NifTAL COURSE OUTLINE

Exercise 1	Nodule Collection and Isolation of Rhizobia
Exercise 2	Observing Cultural and Morphological Characteristics of Rhizobia
Exercise 3	Production of Inoculants and Enumeration of Rhizobia
Exercise 4	Enumeration of Rhizobia in Peat
Exercise 5	Test of Genetic Compatibility between Rhizobia and Legumes
Exercise 6	Experiment to test need for inoculation in pots:
	Grain Legumes - Soybeans and Beans
	Experiment to test need for inoculation in soil cores
	Pasture Legumes - Centrosema and Kudzu
Exercise 7	Field Experiment to Evaluate Legume Germplasm, including N_2 Fixation Traits
Exercise 8	Set up Field Trial (one experiment done by each pair of trainees)

APPENDIX VII

Table 1. Growth, nodulation and nitrogen-fixation characteristics among L. leucocephala, L. diversifolia and the hybrid combined over four inoculation treatments.

Leucaena Species	Growth, nodulation and nitrogen-fixing traits.					
	mg N[b] p^{-1}	shoot dry[b] weight mg p^{-1}	TNA[c]	SNA[d]	nodule dry weight mg p^{-1}	nodule number p^{-1}
L. leucocephala	14.78 a[e] (0.81)	701.75 a (94)	2.95 b	15.98 b	40 a	18.25 b
L. diversifolia	7.39 b (0.54)	291.06 c (28.3)	1.91 c	21.71 a	21.69 b	10.31 c
Hybrid	14.78 a (0.65)	610.31 b (54.5)	3.72 a	17.27 b	47.19 a	25.4 a

[b] Figures in parentheses show data of the uninoculated controls (not included in statistical analysis).
[c] TNA, total nitrogenase activity (micromoles ethylene produced $p^{-1}h^{-1}$).
[d] SNA, specific nitrogenase activity (micromoles ethylene produced g^{-1} nodule fresh weight h^{-1}).
[e] Means having the same letter within a column are not significantly different Duncan's Multiple Range Test ($\alpha = 0.05$).

APPENDIX VII (continued)

Table 2. Growth nodulation and nitrogen-fixation characteristics among the single and mixed strains inoculations combined over the three leucaenas.

Rhizobium inoculation	Growth, nodulation and nitrogen-fixation traits.					
	mg N p^{-1}	shoot dry weight mg p^{-1}	TNA[f]	SNA[g]	nodule dry weight mg p^{-1}	nodule number p^{-1}
TAL 82	7.4 c[e]	437.6 b	2.8 ab	16.7 a	37.8 ab	19 a
TAL 582	8.6 c	397.4 b	2.2 b	17.4 a	28.8 b	15 a
TAL 1145	18.4 a	700 a	3.4 a	19.9 a	41 a	12 a
TAL 82+582+1145	14.8 b	602.5 a	3.1 a	19.4 a	37.6 ab	17 a

e, f, g see Table 1 for explanations.

APPENDIX VII (continued)

Table 3. Analysis of variance for two yield meansurements of two chickpea types as affected by inoculation rate and soil order.

Source	df	Mean Squares	
		Shoot DW p^{-1}	Shoot Total N (mg p^{-1})
Inoculation rate (A)	5	2.673***	2189.***
Soil type (B)	1	0.498*	272.*
Chickpea type (C)	1	20.151***	3914.***
A x B	5	0.180 NS	219.**
A x C	5	0.198*	323.***
B x C	1	0.004 NS	62. NS
A x B x C	5	0.109 NS	20. NS
Error	46	0.099	50.

*, **, *** Significance levels at the 0.05, 0.01, and 0.001 probability levels, respectively.
NS = Not Significant

APPENDIX VIII

INTERNATIONAL TRAVEL BY NifTAL STAFF

NifTAL had two persons outposted full time. One at the BNF Resource Center in Bangkok, Thailand, and with the Zamare Project, a USAID/Zambia project in Lusaka, Zambia. These outposted staff travelled frequently within their region.

TDY travel of Hawaii-based staff during the year was as follows:

Country	Person	TDYs/person days
Bangladesh	1	2/35
Brazil	2	2/21
Burma	1	1/14
Colombia	1	1/7
Egypt	2	2/6
Honduras	1	1/7
India	4	5/29
Nepal	1	1/3
Pakistan	3	5/99
Philippines	1	1/7
Thailand	6	10/65
Zambia	2	2/97

APPENDIX IX

Visiting Scientists Program

Dennis Osborn	Alafua W. Samoa
Nick Poulton	USP Sua Fiji
Gary Smith	Agri Eng Dept, UH Manoa
Michael Crowfoot	Beverly, CA
John J. Slavics	AT Int'l, Washington, D.C.
Noel Vietmeyer	NAS, Washington, D.C.
Dan Shigeta	CES, Kahului, UH
Robert Meller	NCSU, Raleigh, N.C.
Patrick Flanagan	Fairbanks, AK
Ed Rice	USAID/Wash. Asia/TR/ARD
Irwin M. Pikus	NSF, Washington, D.C.
Peter P. Rotar	Agronomy & Soils, UH Manoa
Tom LaRue	Boyce Thompson Inst. Ithaca, N.Y.
Morton Banks	Kettering Inst., Yellow Springs, OH
Betty Roaul	Kettering Inst., Yellow Springs, OH
Kevin S. Guise	U of Minn., St. Paul, MN
David J. Harris	Cali Colombia
Rosemary S. Bradley	CIAT, Cali, Colombia
Judy Kipe-Nolt	CIAT, Cali Colombia
Pedro Alberto Selbach	Porto Alegre, Barzil
William L. Rungle	Prince George, B.C., Canada
Khader Sudgi	O of Jordan, Jordan
Jen Chuan Yang	Taipei, Taiwan
Davis Deardorff	Las Cruces, N.M.
Allen Lundgren	East-West Ctr, Honolulu, HI
Peter Tom	Wewak, Palia New Guinea
Thornley H. Hite	Prime Ministers Off. Honiara, S.I.
Peter Gresshoff	Australia National Univ.
Dieter Mueller-Dombois	UH Manoa
Maimunah Binte Morshi Di	East-West Ctr, Honolulu, HI
Vic Phillips	East-West Ctr, Honolulu, HI
Dennis Grossmhn	East-West Ctr, Honolulu, HI
Ming-Pi Mi	Dept of Genetics, UH Manoa
S. Patil	Plant Pathology, UH Manoa
Yoshiya Asano	Univ. of Nilton at Mishima
Mark Smith	AID/India, New Delhi
Jim Harder	USDA Univ. of Ill. Urbana, ILL
Gary Stacey	Univ of Tenn. Knoxsville TN
Takashi Ozawa	Univ. of Osaka Prefecture, Japan
Kikao Kumuzawa	Tokyo, Japan
Yasuhiro Arima	Tokyo Univ, Fuchucity, Tokyo
Y. Maruyama	Univ. of Tokyo, Tokyo
Takuji Okyama	Univ. of Nagata, Japan
Shigenao Kawai	Univ. Nigata, Japan
John Strieter	Univ/OARDC Wooster, OH
Masuro Yamaguchi	Osaka, Japan
Alvaro Parra	UH, Manoa

APPENDIX IX (continued)

Visiting Scientists Program

Bob Lowen	UH, Manoa
John Brolman	Univ. of Florida, Fort
Cris Escano	PCARRD, Los Banos, Laguna, Phil.
Ramon Valmayor	PCARRD, Los Banos, Laguna Phil.
Tejpal Gill	AID/S&T/AGR Washington, D.C.
Wallace G. Johnson	Raleigh, N.C.
James W. Jones	Univ. of Florida, Gainesville, FL
Robert Rorre	UN Univ Shibuya, Japan
Lowry Harper	Wathinsville, GA
Robert A Stewart	Collingwood, Australia

Visiting Citizen Program

Glenn A. Dodge	Vivian L.C. Anderson
Robert C. Brown	William Frederick Johnson
Jaye S. Tsunoda	Howard Nakamura
Paul Mackey	Fred Matsumoto
Linda Newton	Bob Agres
Mr. & Mrs John Musser	Alvin T. Onaka
Robert Musser	Jean Nishida
Kevin Connerton	Jackie D. Johnson
Tom Sparling	Paul D. Sullivan
Maryloue Wendleng	Thom Aewaele
Larne Byrnes	Vickie Sigman
Clement Chan	Bonnie Tuell
Robert & Eleanor Lee	Carl Myatt
Joel Myhand	Nick Panhoff
Sirl Myhand	Besie Tanaka
Isami Wakoships	Dorothy Myhand
Lesley Hill	Susan Hamilton
Val Ah Loy	Vida Sonoling
Isabel Pires Chung	Ruth L. Toreo
Marion Miyabuchi	Betty Oyama
Alfred Hew	Beatrice H. Kailiponi
David Cup Choy	Clyde Kobayashi
Ed Otsu	Stoney Otsu
Larrayne Otsu	Clayton Carvalho
Jerry Wallace	Lisa Mannion
Liz Musels	

APPENDIX X

NifTAL Publications (1975-1985)

Akyrampong, M., 1980. The Effect of Different Strains of Rhizobium and Phosphorus Levels in Mungbean (Vigna radiata) and Soybean (Glycine (max). M.S. Thesis, Univ. of Hi., 65 pp.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Dowdle, S.F., 1984. China's Technical Contract System: Linking Agricultural Technology to Economic Incentives. Agron. Abst. p. 40.
Dowdle, S.F. and B.B. Bohlool, 1984. Predominance of Indigenous Fast-Growing Rhizobium japonicum in a Soybean Field in the People's Republic of China. Agron. Abst. p. 185.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Halliday, J., 1984. Guidelines for Collecting the Root Nodules of Leguminous Trees. In P. Huxley et al (Eds.) A Manual of Methodology for the Exploration and Assessment of Multipurpose Trees (MPTs). ICRAF, Nairobi.

Halliday, J., 1984. Methods for Assessing Rhizobia and VA Mycorrhizae for Multipurpose Trees. In P. Huxley, et al (Eds.) A Manual of Methodology for the Exploration and Assessment of Multipurpose Trees (MPTs). ICRAF, Nairobi.

Halliday, J., 1984. Principles of Rhizobium Strain Selection. In M. Alexander, (Ed.)

Biological Nitrogen Fixation. Plenum Publ, Corp. p. 155-171.

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

Halliday, J., 1984. Register of Nodulation Reports for Leguminous Trees and Other Arboreal Genera with Nitrogen Fixing Members. Nitrogen Fixing Tree Res. Reports Vol. 2.

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

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

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

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

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

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

Harris, S.C., 1982. Internationally Sponsored Development of Biological Nitrogen Fixation Technology. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a

Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

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

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

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

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

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

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

Keyser, H.H., M.J. Sadowsky, and B.B. Bohlool, 1983. Fast-Growing Soybean Rhizobia. In World Soybean Research Conference III: Proceedings (Ed Richard Shibles), Westview Press. 926-934.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Reyes, V.G., Somasegaran, P., and D.N. Munns, 1978. Catalog of Selected Strains from the NifTAL Rhizobium Collection. NifTAL Project, 16 pp.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Whitney, A.S., 1982. The Role of Legumes in Mixed Pastures. In P.H. Graham and S.C. Harris (Eds.) Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical, 768 pp.

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

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

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

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

Woomer, P., Guevarra, A., and K. Stockinger, 1978. Winged Bean Investigations at NifTAL: Observational Garden Rhizobium Strain Testing and Response Across a Liming Gradient. In. The Winged Bean: Papers Presented in the 1st International Symposium on Developing the Potentials of the Winged Bean. Philippine Council for Agriculture and Resources Research, 448 pp.

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

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

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

Zaroug, M.G. and D.N. Munns, 1980. Influence of Phosphorus and Sulfur Nutrition on Composition o Clitoria ternatea L. Plant and Soil 55:251-258.

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

Conference Presentations (1985 only)

Dowdle, S. and B.B. Bohlool. The Competitive Advantage of Indigenous Fast-growing Soybean Rhizobia. 10th North American Rhizobium Conference, Wailea, Hawaii. Conference Book: p. 34.

Singleton, P.W. and J.W. Tavares. Effect of P or Nodulation and Rhizosphere Colonization of Soybeans by Rhizobium japonicum. American Society of Agronomy Annual Meeting, Chicago, IL, Agronomy Abstract, p 162.

Singleton, P.W. and J.W. Tavares. Response to Legume Inoculation as Affected by Native Rhizobial Populations. 10th North American Rhizobium Conference, Wailea, Hawaii. Conference Book: p. 55.

Somasegaran, P., V. Gurgun, and H.J. Hoben. Effect of Rhizobium Inoculation Rates on Nitrogen Fixation and Competition for Nodulation on Chickpea (Cicer arietinum L.). 10th North American Rhizobium Conference, Wailea, Hawaii. Conference Book: p. 69.

van Kessel, C. and P.L. Nakao. The Use of ^{15}N-depleted Ammonium Sulfate for Estimating N_2-Fixation by Leguminous Trees. 10th North American Rhizobium Conference, Wailea, Hawaii. Conference Book: p. 43.

van Kessel, C., P.W. Singleton, and H. Hoben. Enhanced N-transfer from a legume to a non-legume by vesicular-arbuscular mycorrhizal fungi. 10th North American Rhizobium Conference, Wailea, Hawaii, Conference Book: p. 72.