PD-AAY-496

MEMORANDUM

Date 30 Sep 88

TO: PPC/CDIE/DI, Tina Wilson/Acquisitions - 209 SA-18

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

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

Attached is one copy of the following article with the supporting contract/grant number:

1987 Annual Report - Improved BNF through Biotechnology/NIFTAL Project

Contract/Grant Number: DAN-4177-A-00-6035-00

Project: 936-4177

Attachments: a/s

cc: T.Gill

D. Bathrick

PD-AAY-498

1987
Annual Report
U. HAWAII - NITAL

PD-AAY-198

NifTAL Report 1987

Improved BNF Through Biotechnology/ NifTAL Project

USAID CONTRACT DAN-4177-A-00-6035-00

Dr. B. Ben Bohlool, Principal Investigator

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

United States Agency for International Development

STATISTICAL SUMMARY

Title: Improved BNF Through Biotechnology

Contract Number: DAN-4177-A-00-6035-00

Principal Investigator: B. Ben Bohlool

Address: NifTAL Project and MIRCEN
1000 Holomua Avenue
Paia, Maui, HI 96779-9744 USA

Contractor: University of Hawaii
Department of Agronomy and Soil Science
College of Tropical Agriculture and Human Resources

Contract Period: 10-1-86 to 12-31-87

Reporting Period: 1-1-87 to 12-31-87

Contract Funds: $1,650,000

Project Office: S&T/AGR/RNR

Project Monitor: Dr. Lloyd R. Frederick
S&T/AGR/RNR, SA-18
Agency for International Development
U.S. International Development
Cooperating Agency
Washington, D.C. 20523

TABLE OF CONTENTS

Title Page
Statistical Summary ---------- 2
Table of Contents ---------- 3

OVERVIEW:

Background ---------- 5
Summary of Accomplishments ---------- 6
Acknowledgements ---------- 7

OUTREACH:

Training ---------- 10
Rhizobium Technology/Inoculant Production Course
Visiting Scientist Program
Graduate Students

Outreach ---------- 12
Worldwide Rhizobial Ecology Network
Technical Services
Material and Service Provision
Information Services

RESEARCH:

Genetic Technologies for Improvement of *Rhizobium*/Legume Symbiosis for Crops and Trees (Genetic Technologies) ---------- 19

Development of Methodologies for Monitoring Microorganisms Introduced into the Environment (Minotoring Methods) ---------- 20

Environmental Constraints to Maximal Performance of Biological Nitrogen Fixation (Environmental Studies) ---------- 22

Rhizobium Ecology ---------- 33

Inoculant Production Research and Transfer (Inoculant Production) ---------- 37

APPENDICES

I. Publication List 1975 - 1987
II. Abstracts of Work Published during 1987
III. Abstracts of Meeting Papers and Posters Presented during 1987
IV. Grants and Funding Sources
V. Visitors List: BNFRC (Bangkok); NifTAL (Maui)
VI. Inoculant Production Course Contents
VII. Staff International Travel
VIII. Experimental Materials Kit Contents
IX. Personnel List

OVERVIEW

OVERVIEW

Background

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

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

From June 16, 1982, through September 30, 1986, (USAID Contract DAN-0613-C-00-2064-00), NifTAL continued to develop appropriate BNF technologies for tropical agriculture as well as test these technologies in developing countries. Also during this period, technology transfer and training activities increased--particularly those related to production and use of inoculants.

Beginning in 1987, NifTAL began its second decade under a service agreement known as Improved BNF Through Biotechnology. This award was given based upon the continuing and expanding need for NifTAL's services and acknowledging the excellent work done by the project during its first ten years.

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

Objectives:

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

Specific objectives were:

to amass and maintain a Rhizobium germplasm resource comprised of rhizobial strains which effectively nodulate agriculturally important legumes under the conditions prevailing in developing countries.

to establish an antisera bank for identifying Rhizobium so that the yield benefit resulting from the inoculation of agriculturally important tropical legumes could be measured.

to develop systems for improved inoculant delivery and for insuring dependable, effective nodulation in the field.

to identify management techniques for increasing nitrogen fixation in the field.

to determine the effects of soil inertility on symbiotic performance of legumes.

to provide economic information on the cost of BNF technology relative to that of nitrogen fertilizers.

to provide regional and in-country support to legume programs in developing countries.

to train professional researchers, technicians, and extension workers in the development and implementation of legume-based BNF technologies.

SUMMARY OF ACCOMPLISHMENTS

During 1987, NifTAL continued to meet its outreach objectives of providing training, technical assistance, material services, and information, while continuing to strengthen its research objectives.

Scientific experiments were carried out in some new areas. For example, screening was initiated to determine whether strains of rhizobia differed in their ability to retain symbiotic plasmids at high temperatures. Distribution of inoculants in the tropics will require that temperature does not adversely affect either viability or symbiotic characteristics of inoculant strains.

In a cooperative effort with Indian scientists, high quality antibodies were developed against strains of chickpea rhizobia which had not previously elicited an immune response in rabbits. These antibodies will now permit scientists in South Asia and the Middle East to monitor the success of their chickpea inoculants in the field.

Efforts continued to develop methods to measure N_2 fixation by trees. Natural abundance methods offer promise if careful selection and monitoring of reference species are undertaken. This research has profound implications for the study of N-cycling in agroforestry and alley cropping systems.

The physiological basis of NO_3 assimilation by soybean and *Phaseolus* was compared in an effort to explain the apparent low N_2 fixation potential of *Phaseolus*. Results indicate that a screening method may be developed to identify germplasm with greater N_2 fixation potential.

Models predicting the occurrence and diversity of rhizobial populations based on environmental data were developed. Results, after future refinement, will allow scientists to construct maps of populations. This predictive capability will identify ecosystems where legume inoculants will be required in crop production.

Details of research work appear later in this report. Appendix I is a complete listing of NifTAL staff publication from 1975 to 1987. Abstracts of both published work and presented papers and posters are Appendix II and Appendix III, respectively.

In the outreach area of training, NifTAL instructors and course planners cooperated with FAO and the Department of Agriculture, Bangkok, Thailand, to host a *Rhizobium* Technology and Inoculant Production Course. The course was offered to 11 scientists from 10 countries. Five persons--from Ghana, the Philippines, Nepal, and Western Samoa--spent time at NifTAL headquarters in Maui conducting research and learning about NifTAL's facilities and objectives. As well, six graduate students worked toward M.S. and Ph.D. degrees. One successfully completed his Ph.D., and another his M.S. The BNF Resource Center (BNFRC) in Thailand trained two additional students during the year. Appendix V lists persons who visited both the BNFRC and NifTAL Headquarters.

Highlighting the networking activities this year was the initiation of the Worldwide Rhizobial Ecology Network. This network is an upward step from NifTAL's INLIT (International Network of Legume Inoculation Trials). This network is funded in part through a grant from the National Science Foundation. Twenty-two collaborators from twenty countries are conducting carefully designed experiments with a goal of producing a predictive model of legume response to inoculation.

Technical assistance was requested from several countries which were visited by NifTAL staff (Appendix VII contains a listing of international travel by Project staff). Advise was given on questions ranging from how to produce legume inoculants to the types of tree species most effective for reforestry and agroforestry projects. Materials were provided in the form of seed, inoculants, and pure cultures. Two hundred and twenty-five packages of inoculant were sent to 25 countries in response to 34 requests, as well as 1246 cultures being sent in response to 126 requests from 40 countries.

Information was provided through contacts with NifTAL scientists and through activities of the Communication Section. An issue of the BNF Bulletin and Number 2 in the Illustrated Concept Series were published and distributed to 1750 persons in more than 100 countries. A newsletter was initiated called the WREN Newletter; this was distributed to nearly 500 persons. Nearly 1300 other documents were distributed to requesters during the year. Two audio-visual programs were also produced.

Acknowledgements

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

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

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

OUTREACH

TRAINING

Rhizobium Technology/Inoculant Production Course

An International Training Course on *Rhizobium* Technology and Inoculant Production, supported by FAO/Thailand DOA/BNFRC, was held in Bangkok, Thailand from March 2-27, 1987. A total of 11 scientists from 10 countries participated in the course. Instructors were Nantakorn Boonkerd (BNFRC), Padma Somasegaran (NifTAL), and Heinz Hoben (NifTAL). The Course Contents is attached as Appendix VI. The following persons received training during this course: Kang Jin Cho (Republic of Korea); V.N.P. Gupta (Nepal); Nguyen Hai Nam (Vietnam); Delowara Khanam (Bangladesh); Shahida Nasreen (Pakistan); P. K. Samaradeewa (Sri Lanka); Jusuf Soepriaman (Indonesia); Fe Torres (Philippines); and Li Xin (P.R. of China).

Visiting Scientist Program

Dr. Mohammed Asgar (University of the South Pacific, Alafua Campus, Western Samoa) spent three days at NifTAL (Feb. 11-13, 1987) to familiarize himself with the activities of the NifTAL Project and to gain some knowledge of *Rhizobium* inoculum preparation and the handling of inoculum for experimentation.

Mr. Robert Abaidoo (Ghana), a researcher and lecturer at the University of Science and Technology, worked at NifTAL from October 1986 to December 1987. He was sponsored by a fellowship from the Atomic Energy Agency and funded by the National Academy of Sciences - National Research Council. His work focused on the use of ^{15}N methods in quantifying nitrogen fixation in tropical legumes. He conducted three greenhouse experiments with Dr. Chris vanKessel. He also conducted field experiments with other NifTAL scientists while working with an important African pulse legume crop, Bambara groundnut and Lima bean. His work was presented in a poster at the 11th North American *Rhizobium* Conference in Quebec, Canada.

Dr. J.K. Ladha (Philippines) visited NifTAL on September 4, 1987. The objective of his visit was to become familiar with NifTAL's facilities and with ongoing work here.

Agnes Padre (Philippines) interned at NifTAL from August 20 to September 4, 1987. During her two week stay she consulted with NifTAL staff on general techniques in *Rhizobium* technology.

Dala Ram Pradhan (Nepal) arrived June 10, 1987 and departed June 23, 1987. He learned how to produce inoculants on a small scale and participated in the following experiments: 1) Estimation of rhizobial populations in a mixed pasture of grass and legumes and 2) Pasture improvement through legume introduction. Details are shown in the research report.

Graduate Students

Taufiqul Aziz (Bangladesh) completed his dissertation entitled: **Use of Vesicular-Arbuscular Mycorrhizal Fungi for Establishment of Effectively Nodulated Legumes on Moderately Weathered Oxisol Subjected to Simulated Erosion.** He defended his thesis in June 1987.

Maria Luz Caces (Philippines) has been accepted as a Ph.D. candidate. She is working with *Rhizobium phaseoli* and has so far characterized six strains inoculated with five different strains of *Bradyrhizobium japonicum*.

Thomas George (Kerala, India), Ph.D. candidate, completed his course work and comprehensive examination. He also completed the following experiments: 1) a growth chamber experiment comparing the nitrogen assimilation characteristics of *Phaseolus vulgaris* and soybean at two temperatures (18 and 25 degrees C); 2) two field experiments investigating the effects of elevation associated changes in temperature and levels of nitrogen supply (9, 120, and 900 Kg N/ha) on growth, yield, phenology, N_2-fixation and mineral N assimilation in *P. vulgaris* and soybean. ^{15}N isotope of nitrogen was used to determine N_2-fixation and fertilizer use efficiency. Chemical analysis and statistical analysis are in progress.

Naseer Mirza (Pakistan) joined NifTAL in August 1985 as a Master's student. His research work with native rhizobia from soils included two projects: 1) investigating patterns of effectiveness of six agroclimatic locations on Maui and 2) chlorophyll estimation in soybean plant as a quick bioassay for estimating the N_2-fixing potential. He found that chlorophyll content was highly correlated to shoot dry weight, N content, nodule dry weight and nitrogenase activity in soybean. After completing his degree he departed in December 1987.

Janice Pinchin Thies (USA) completed six of eight field studies designed to provide a database for the development of a model to predict legume response to rhizobia inoculation. Data from the first three field experiments were used to develop a preliminary working model to predict mean percent increase n yield due to inoculation as a function of soil rhizobia number. The model was presented in a talk given at the 11th NARC in Quebec City, Quebec, Canada. Data from the remaining three completed field experiments were analyzed and incorporated into the preliminary working model. Field sites were prepared for the final two field studies in this series. Thies also attended a 4-week short course sponsored by the IBSNAT Project on "Collection of Minimum Data Sets for Crop Modelling."

Tanya Thongnunui (Thailand) joined the BNFRC May 1, 1987, under the NSF Project. She worked on the response of legumes to inoculation in relation to the population of indigenous rhizobia in two locations, North and Northeast Thailand. MPN plant infection counts of soils have been completed.

In Thailand, two students each from Chulalaongkorn University (April 15 - May 15, 1987) and King Mongkut Institution (May 4 - May 29, 1987) came to the BNFRC for training.

OUTREACH

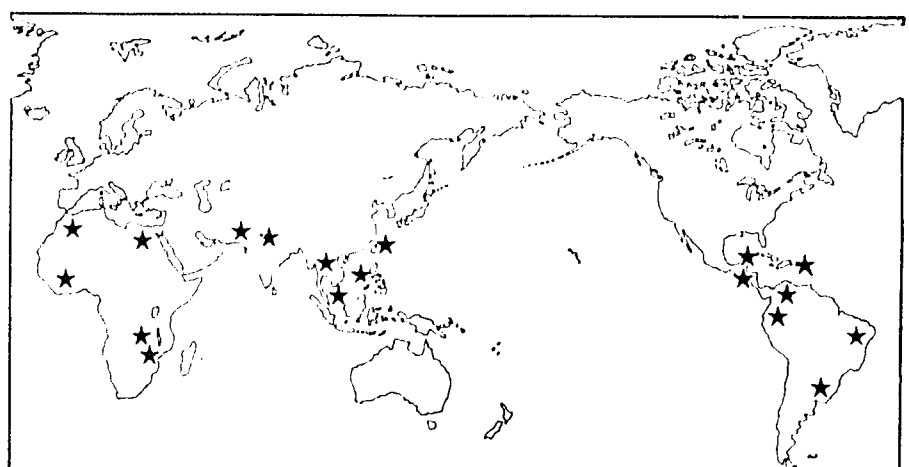
Network

NifTAL began its networking activities with the International Network of Legume Inoculation Trials (INLIT) in 1979. This network was initiated to determine whether, under defined conditions, a yield increase could be obtained through legume inoculation using the state-of-the-art. Growing out of this concept is a more complex and ambitious experimental network known as the Worldwide Rhizobial Ecology Network (WREN) which is funded in part through a grant from the National Science Foundation.

Twenty-two carefully selected collaborators (see Figure 1) are conducting experiments along with NifTAL to obtain the large database required to construct and verify a predictive model of legume response to inoculation. WREN members, scientists linked by their interest in the ecology of *Rhizobium* in tropical soils, have contributed to the standard experimental protocols for the laboratory and greenhouse studies developed by NifTAL. Another phase of the experiment will be field trials using legumes of choice.

In 1987, experimental kits (contents listed in Appendix VIII) for evaluating rhizobial populations in soil and for conducting standardized field tests of plant response to inoculation were distributed. Soil samples, climate data, and characterization of native rhizobia at collaborators' test sites were received. Most collaborators used the trials as part of their graduate student training program further enhancing the networking potential. Greenhouse and field test portions of the protocols have been initiated by most collaborators. Their results will be incorporated with data generated in Hawaii to derive the plant response to inoculation model.

Figure 1. WREN Collaborators



Ricardo S. Araujo
Goiana, Brazil

Robert Abaidoo
Kumasi, Ghana

Dr. Kauser Malik
Faisalabad, Pakistan

Dr. Carlos Labandera
Montevideo, Uruguay

Dr. Rosemary Bradley
Cali, Colombia

Dr. Juan Carlos Rosas
Tegucigalpa, Honduras

Dr. Eduardo Schroder
Mayaguez, Puerto Rico

Dr. Vernon Chinene
Ronnie Nyemba
Lusaka, Zambia

Dr. Carlos Ramirez
Turrialba, Costa Rica

Dr. S. V. Hegde
Bangalore, India

Dr. C. C. Young
Taichung, Taiwan

Dr. Paul Davis
Marondera, Zimbabwe

Sra. Consuelo Estevez
Quito, Ecuador

Dr. Zulkifli Shamsuddin
Sedang, Selangor, Malaysia

Dr. N. Boonkerd
Dr. P. Wadisirisuk
Bangkok, Thailand

Dr. Hassan Moawad
Dokki, Egypt

Dr. Ali Hilali
Rabat, Morocco

Dr. Erlinda Paterno
Dr. Mercedes Ulmali Garcia
College, The Philippines

Some results of work done are development of quantitative models describing the number and species diversity of rhizobia in tropical soils; creation of a computer program "Most Probable Number Enumeration System (MPNES)" which generates solutions for population estimates of MPN data of plant infection counts; completion of statistically evaluating more than 500 plant infection counts for accuracy and reliability of technique; monitoring survival of three ecologically identifiable strains from each of six species of rhizobia at fourteen ecologically distinct sites; and completion of eight field experiments on Maui and two in the Philippines testing the response to legume inoculation

Ecological work in 1988 will center on defining and testing models, defining the role of specific host plants on rhizobial survival; determining whether natural plant communities can be altered by the introduction of exotic microorganisms; and development of a bioassay to define effectiveness of a soil rhizobial population. Crop response work will be carried out through field trials in Hawaii, continued technical and materials support of collaborating scientists, and development of models describing (1) how environment and native populations of rhizobia affect the establishment of inoculant strains on root systems of plants and (2) how crop history and management affect soil populations of rhizobia. A meeting is being planned for all collaborators in late 1988 or early in 1989. This meeting will be used to develop a final ecology and crop response model for publication

Technical Services

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

Bangladesh: Technical assistance was given to organize BNF Training for the country. Another contact was made which resulted in a Buy-in for a 1988 4-week review of Agricultural Research Project. Phase II.

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

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

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

Haiti: A proposal and revised proposal were submitted to the USAID/Port-au-Prince, Haiti entitled "Development and Evaluation of Rhizobia, Frankia, and VA Mycorrhizal Inoculants to Enhance Survival and Growth of Agroforestry Outreach Project Trees." Also, 100 bags of inoculant for *Leucaena leucocephala* and *Gliricidia sepium* were sent to the Agroforestry Outreach Project.

India: Continued the close collaboration with Indian scientists in the Indo-US Scientific initiative. A complete report on this initiative is available separately.

Indonesia: A staff member made contact with George Like (USAID/Jakarta) who had just arrived from Guatemala. They discussed the possibility of NifTAL providing training and

research support for the Applied Agricultural Research Project.

A review was made of commercial forestry activities in East Kalimantan (Samarinda) Indonesia, and about research on ectomycorrhizae for Dipterocarps being conducted there. The nodulation status of *Acacia mangium* seedlings being used in reforestation project was assessed.

Pakistan A NifTAL consultant made contacts for an 8-week consultation to be held in 1988 regarding the national BNF program and inoculant production.

Philippines: A proposal was developed to assess need for inoculant production and extension activities for AID Manila. This proposal is pending as of Dec 31, 1987. A series of field experiments are being carried out in as part of collaborative research between NifTAL and Philippine Council for Agriculture and Resouces Research and Development, Camarines Sur State Agricultural College, and Marianos Marcos State University.

Sri Lanka: An initial visit resulted in a buy-in for a 6-week consultancy on the country's legume inoculation program and inoculant production.

Thailand: Cooperation continued between Thai and NifTAL scientists with the BNF Resource Center as a central point of contact.

Material Services

During 1987, materials were provided to requesters in many countries. Scientific support was given in the form of *Rhizobium* inoculants, pure cultures, antisera, and seed. Inoculants were generally requested for experimental purposes; cultures for experimentation and inoculant production; antisera for identification using the fluorescent antibody technique; and seed for collaborative experiments.

Antisera requests were received from researchers in many countries, for example, Nigeria, Thailand, Nepal, Pakistan, Belgium, and Great Britain. In addition to replenishing those held in stock, two new antisera were produced and added to the antiserum bank. These new antisera are TAL 943 and TAL 1797 (P. vulgaris). See the NifTAL Antisera Catalog for a complete list of antisera available.

Requests were made for seed of a variety of legume species types, i.e., tree, forage, and grain/pulse. Increases were noted in requests for leguminous tree species reflecting the current interest in agroforestry and reforestation.

Many requests were made for inoculants. In summary, 225 packages of peat inoculant (20.25 kg) was sent out to 25 countries in response to 34 requests. Produced materials list and completed development of fermenters for Mt. Makulu Inoculant Production Station (Zambia). Listed in Table 1. below is a synopsis of *Rhizobium* cultures distributed in 1987.

TABLE 1. NifTAL Rhizobial Germplasm Resource Services

Country	Requests	# of Cultures
Argentina	1	8
Austria	1	3
Bangladesh	3	50
Belgium	1	6
Brazil	2	39
Burkina Faso	1	3
Canada	5	18
Colombia	1	23
Costa Rica	3	31
Ecuador	3	29
Egypt	1	23
England	6	41
France	5	26
Ghana	2	36
Honduras	2	30
India	13	150
Malaysia	2	31
Mexico	1	2
Morocco	1	23
Nepal	3	32
Netherlands	1	2
Pakistan	7	56
Philippines	9	116
Peoples Republic of China	1	23
Saudi Arabia	1	24
Scotland	1	3
Sri Lanka	2	21
Sudan	1	12
Sweden	2	15
Switzerland	1	2
Taiwan	1	24
Thailand	4	42
Turkey	1	3
USA	26	128
Uruguay	1	23
Venezuela	3	46
West Bengal	1	17
Zaire	1	3
Zambia	2	32
Zimbabwe	3	50
40	126	1246

Information Services

In 1987, the NifTAL Communication Section produced a variety of publications. These ranged from continuing publictions like the Annual Reports, BNF BULLETIN newsletter, and the second in the Illustrated Concepts in Biotechnology Series, to new publications like the WREN Newsletter. In addition, ten scientific posters, and three sets of slides were assembled for special purposes. Tours were conducted for large groups like the President's Club of the University of Hawaii Foundation, and school classes and small groups of visiting scientists for the USA and foreign visitors. For the fourth year, NifTAL cooperated with Maui Community College to host and sponsor the World Food Day Teleconference as the sole site receiving the conference in the State of Hawaii.

More than 800 requests were received for reprints, NifTAL Publications, and addition to the NifTAL mailing list. An address dissemination update was mailed with the BNF BULLETIN which contained a request for information about respondent's scientific discipline and extent of interest in BNF. A good response was received. Also, begun in late 1987 was computerization of the NifTAL Document Collection. This database bibliography will be used as a readily accessible source of information for both NifTAL staff and developing country researchers. It is expected to be completed in early 1988.

A synopsis of publications produced and information requests filled follows:

Information Publications

1985 Annual Report

Printed Materials:

1986 Annual Report/Final Report 1975-1986 (68 pages).

Illustrated Concept Number 2, "A Small Glass Fermenter for the Production of Rhizobium Inoculum." (1-page)

BNF Bulletin, Volume VIII, Number 1, Summer 1987. 12-page plus 4 page Continuing Bibliography #14 (attached)

WREN Newsletter, Number 1, 1987. 8-page.

Audio-Visual:

"Humans Need Protein for Life", audio-visual program, 8 minutes, 61 slides.

Update "What is NifTAL?", audio-visual program, 12 minutes, 78 slides.

Food Legumes of The Tropics, slide set with text, 55 slides (author, P. Woomer)

Information Dissemination

NifTAL:

Reprints (NifTAL Staff):	339
Document Collection:	155
Miscellaneous publications:	152
Methods in Rhizobium-Legume Technology:	88
BNF Bulletin, Vol. VIII, Number 1:	1750
WREN Newsletter, Number 1, 1987:	490
Illustrated Concept #2, "A Small Glass Fermenter for the Production of Rhizobium Inoculum:	1700
1986 Annual Report/Final Report 1975-1986:	200

Thailand:

Reprints:	12
Legume Inoculant and Their Use (in Thai) :	7
Methods in Legume-Rhizobium Technology:	1
Miscellaneous Publications:	17
Document Collection:	10

RESEARCH

GENETIC TECHNOLOGIES

Loss of Symbiotic Plasmids from Rhizobia in Inoculants Exposed to High Temperatures

Symbiotic genes for nodulation (nod) and nitrogen fixation (nif-fix) are harbored on the symbiotic plasmids of many *Rhizobium* spp. Without the physical presence of the symbiotic plasmid, the microsymbiont will not nodulate the host legume. Non-nodulation due to the loss of symbiotic plasmids in cells grown in broth cultures incubated at 37 degree C or greater has been demonstrated with many *Rhizobium* sp. We investigated the influence of high temperature and moisture stress on the symbiotic plasmids of *Rhizobium* sp. cultured in peat. This investigation was of practical significance because it is not unusual for rhizobial inoculants to be exposed to adversely high temperatures under tropical conditions.

We screened six strains of *Rhizobium leguminosarum* bv. *viceae* for loss of symbiotic plasmids. All strains carried transposon (Tn5) insertions (with resistance to kanamycin) on the symbiotic plasmids and their plasmids were visualized by agarose electrophoresis. Peat inoculants of the strains were incubated at 28 degree C and 37 degree C under normal and stress moisture levels. Loss of symbiotic plasmids was monitored by plate counts on antibiotic medium, plant infection counts and agarose electrophoresis.

Results showed that high temperature and moisture stress decreased viability of the rhizobia in the inoculants. With some strains the plate counts were significantly higher than the plant infection counts indicating that the viable cells in the inoculant were not capable of nodulation. However, the inability to nodulate was not due to loss of symbiotic plasmids as confirmed by plate counts on antibiotic medium and agarose electrophoresis. It is possible that the inability to nodulate was due to deletions in the symbiotic plasmid rather than its loss. We concluded from our data that *Rhizobium* sp. are unlikely to lose their symbiotic plasmids in peat inoculants exposed to high temperature and moisture stress.

Host control of nodulation in a split-root system in response to mutants of *Bradyrhizobium japonicum*

The soybean host plant regulates the development of root nodules which house its microsymbiont *B. japonicum*. The host responds within hours of initial infection to inhibit nodulation on developmentally younger regions of the root (Pierce and Bauer, 1984). This regulation response explains the common clustering of nodules in the crown area of the soybean root system, and has profound implications on the host's role in determining the outcome of strain-strain competition patterns.

In a split-root system, prior inoculation of one root-half suppresses secondary nodule development on the other half (Kosslak and Bohlool, 1984). We used a split-root system to examine various mutant strains of *B. japonicum* for their ability to elicit this suppression response and to compete for nodule sites. The split-root growth system (Singleton, 1983) is useful for reasons: 1) it allows us to identify nodules of the same strain originating from separate inoculations; 2) we are able to quantify nodules occupied by isogenic strains; and 3) we can isolate host mediated competition patterns from those determined by strain differences in rhizosphere competence. While the signals from microbe to host which trigger the regulation response have not been

identified, this type of screening of parents and mutants may help to identify the factors which confer competitiveness to a strain by their ability to induce the regulation response.

Mutants with ineffective nodulation phenotypes developed by UV-exposure (Dr. W. Brill, University of Wisconsin) or Tn5 insertions into the *nif*A or *nif*H genes (Dr. G. Stacey, University of Tennessee) formed as many nodules as their parents when the opposing half-root systems were inoculated simultaneously with a standard competent strain (USDA 110). However, when we inoculated half-root systems with the wild-type and mutant strains one week prior to inoculation of the opposing root system by USDA 110, the ineffective mutants did not suppress secondary USDA 110 nodules to the same extent as the parent strains did. When photosynthate was limiting due to low light conditions, a mutant strain carrying a Tn5 insertion in the *nif*KD structural gene (G. Stacey) formed only few nodules even when applied to a half-root system one week prior to inoculation of the second half-root with USDA 110 (no suppression). Under increased light, early inoculation with this mutant resulted in the supression of primary nodules, and secondary inoculation by USDA 110 was significantly suppressed. Under either light condition, this strain formed only few nodules when simultaneously inoculated on half-root systems with either USDA 110, or its wild-type parent, or another isogenic mutant on the other side. Pot studies confirmed the slow-to-nodulate phenotype of the *nif* mutant. In our studies, the various mutations resulting in ineffective nodulation did not affect the strains' competition patterns, regardless of the site or nature of the mutation. In contrast, the mutation affecting the strain's virulence, or speed to nodulation, had profound effects on its ability to compete for photosynthate for nodule development.

MONITORING METHODS

INTRODUCTION

Our ability to monitor rhizobia in soils and in root nodules is of paramount importance in determining the success of introducing exotic strains into crop systems. Many constraints limit the precision and reliability of existing technology. Monitoring the fate of fixed and quantifying the amount of N_2 fixed in crop systems also requires improved methodology if we are to understand the full costs and benefits of BNF in agriculture.

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

Several years ago, Indian researchers failed in producing antibodies against several chickpea rhizobial strains in their collection. Several unsuccessful attempts showed that this recalcitrance to produce antibodies was strain specific and not due to animal variation. Since some of these strains are efficient nitrogen fixers their performances under different environmental conditions need to be investigated. Good quality antisera are therefore required to monitor their populations.

The rhizobial strains of chickpea and other rhizobia used in our study are shown in Table 1. The study was focused mainly on TAL 1791.

Searching for similarities between recalcitrant strains: The strains listed (Table 1) were characterized in regard to their intrinsic resistance to 10 antibiotics (IAR) at various levels of concentration and by plasmid analysis. Small differences were observed in the IAR patterns of all recalcitrant strains with the exception of TAL

1109 and TAL 1790 which showed identical resistance patterns to all antibiotics. The plasmid profiles were different in all strains including TAL 1109 and TAL 1790. This confirms that we have no strain duplication but probably closely related strains. It is expected that future work on lipopolysaccharide analysis and protein profile determinations will reveal further features unique to recalcitrant strains.

Table 1. Recalcitrant and non-recalcitrant strains of rhizobia.

Designation	Immunogenicity	Host Legume	Rhizobial species
TAL 1791	recalcitrant	*Cicer arietinum*	*Rhizobium sp.*
TAL 1793	recalcitrant	*Cicer arietinum*	*Rhizobium sp.*
TAL 1109	recalcitrant	*Cicer arietinum*	*Rhizobium sp.*
TAL 1111	recalcitrant	*Cicer arietinum*	*Rhizobium sp.*
TAL 1790	recalcitrant	*Cicer arietinum*	*Rhizobium sp.*
2 le	non-recalcitrant	*Cicer arietinum*	*Rhizobium sp.*
TAL 102	non-recalcitrant	*Glycine max*	*Bradyrhizobium japonicum*
TAL 182	non-recalcitrant	*Phaseolus vulgaris*	*R. leguminosarum* bv. *phaseoli*

Immunoresponse based on different inoculation schedules and antigen concentrations: Under the assumption that different injection schedules may produce antisera of different antibody levels, six different schedules were used with the antigens of TAL 1791, TAL 102, 2 le, and TAL 182. The schedules included single and combinations of multiple injections by various routes e.g. intramuscular, intravenous, subcutaneous and intra foot pad. The agglutination titres of the resulting antisera were not significantly different. However, the results of the foot pad injections still remain to be determined.

In another study two antigen concentrations of TAL 1791, full strength (1×10^9 cells/ml) and reduced strength (5×10^7 cells/ml) were administered to different animals. Each antigen treatment was duplicated with a different injection schedule. In all treatments similar low titres (100) resulted; however, it may be significant that antibody production was induced at such low antigen levels.

Unless the results of the foot pad injections bring new results we are led to believe that injection routes have little influence on antibody production. The fact that antibody production was induced at 1/20 of the regular dose seems to indicate that we should in future lower our antigen concentration.

Antibody production response to mixed strain immunization: This investigation is based on the hypothesis that the mixing of a non-recalcitrant strain of *Rhizobium* with the recalcitrant strain may boost the antibody production against the recalcitrant strain. Table 2 shows the different antigens used with recalcitrant strains TAL 1791 and the resulting titres. The results show that the presence of *Bradyhrhizobium japonicum* TAL 102 significantly boosted the titre of TAL 1791 while the chickpea strain 2 le and the *Phaseolus* strain TAL 182 had no effect.

Selecting alternative methods for antibody titration: Perhaps high agglutination titres do not represent a quality measure of an antiserum. For example, a high agglutination titre need not necessarily indicate strong precipitation band development in gel diffusion. This could be either because a different set of immunoglobulins is involved in the reaction or the sensitivity of the method is so high that a high concentration of antibodies is not required.

Table 2. Agglutination titres of single and mixed strain immunization.

Immunogen(s)	Immunization	antisera			
		2 le	TAL 1791	TAL 102[a]	TAL 182[b]
		agglutination titre			
2 le*	Single strain	1600	----	----	----
TAL 1791**	Single strain	----	400	----	----
TAL 1791 + 2 le	Mixture	200	1600	----	----
TAL 1791 + TAL 102	Mixture	----	3200	3200	----
TAL 1791 + TAL 182	Mixture	----	400	----	400

*non-recalcitrant *Rhizobium* sp (chickpea)

**recalcitrant *Rhizobium* sp (chickpea)

[a] non-recalcitrant *Bradyrhizobium japonicum*

[b] non-recalcitrant *Rhizobium leguminosarum* bv. *phaseoli*

Antisera of TAL 1790 (titre 3200) and TAL 1793 (titre 200) were serially diluted and then reacted with their homologous antigens. TAL 1790 showed precipitin bands up to a 1:2 dilution while recalcitrant TAL 1793 still reacted strongly at a dilution of 1:4. The strains did not cross react.

The immunoblot technique was used to react antisera of TAL 1791 (titre 200), TAL 1793 (titre 400) and 2 le (titre 3200) with 23 rhizobial strains of chickpea which included the recalcitrant strains TAL 1791, TAL 1793, TAL 1111 and TAL 1109 at twofold dilutions from 1:250 - 1:8000. All homologous reactions were equally strong to the highest dilutions. The antibodies of TAL 1793 reacted with all recalcitrant strains present plus three additional ones. The antibodies of TAL 1791 reacted with the same strains and two in addition which reacted up to a dilution of 1:4000 indicating strong simlilarity but not identity. This finding is supported by the plasmid profiles as well as the intrinsic antibiotic resistance patterns as shown earlier. Antibodies of 2 le seemed more specific as it cross reacted with only one (non-recalcitrant) strain.

- Enumeration of Rhizobia From Soil

The reliability of the plant infection technique which is used to test rhizobia in soil, was examined using six legume species and eleven soil serles from four different soil orders (Oxisols, Inceptisols, Ultisols, Mollisols). The statistic generated is the range of transition from all positive growth units to all negative. Greater range of transition indicates low precision. Results indicated that recovery of rhizobia was not affected by large differences in soil type but was significantly affected by species. This study indicates that researchers need to validate their method of plant infection technique but that results between soils and sites can be compared. A publication is in preparation.

The development of a computer program which generates most probable number (MPN) solutions in either tabular form or as individual data allows for increased

flexibility in MPN procedures. More choice in deciding base dilution ratios and replicate number result in increased accuracy of MPN experiments. This computer program will be offered at cost to interested scientists as the Most Probable Number Enumeration System (MPNES).

- Monitoring N_2-Fixation in Fast Growing Trees

An alley cropping experiment was initiated using *Leucaena leucocephala, Gliridicia sepium* and corn. Alley cropping is being promoted as a sustainable, productive alternative to slash and burn and modern high input agricultural systems. This experiment will examine the N economy of the system, especially the system's N contribution to corn. This information is critical in determining the value of one of the major reasons their crop system is being promoted for small farmers. Isotope methods employed in the experiment to follow N in the alley crop system will provide fundamental information on how to best measure N_2 fixation by fast-growing trees.

Analysis of tissue from a series of inoculation experiments conducted in a variety of environments indicate that through careful selection of reference crop and N source, N_2 fixation can be measured through ^{15}N natural abundance methods in tropical environments. These results may, with further study, eventually permit N_2 fixation measurements to be determined without the use of costly enriched ^{15}N isotope.

ENVIRONMENTAL STUDIES

INTRODUCTION

The presence of environmental constraints to BNF is almost universal in farmers fields. Any environmental stress acting on any of the complex processes of the symbiosis will result in reduced amounts of N_2 fixation and yield. The effects of soil stresses such as low P fertility, salinity, drought and acidity can directly affect rhizobia in the soil, in inoculants or at infection sites on plant roots. These same stresses also may affect root growth, susceptibility of infection sites and plant photosynthetic capacity which reduces energy sources for the reduction of N_2 in root nodules. Environmental stress may also come in the form of other microorganisms competing for resources on the root surface and competing for infection sites at the expense of our selected, highly efficient strains. Even soil N availability may be considered a stress upon the symbiosis since the assimilation of mineral N substitutes directly for fixed N and legume culture can therefore deplete soils of N under certain circumstances.

While it is true that we can generally enhance BNF by increasing management inputs to alleviate environmental stress, it is not frequently economic to increase input levels solely to increase BNF output of the system. At this stage of our knowledge we do not have a clear understanding of how or which process in the symbiosis should be improved to tolerate environmental stress. Basic and very feasible research is required to clearly identify specific aspects of the symbiosis which could be manipulated to increase tolerance to specific stresses. We have chosen to examine some specific environmental stresses and conduct research on particular processes in the symbiosis which require a greater understanding before remedies can be proposed.

- *Bradyrhizobium japonicum*-environment interactions: nodulation and interstrain competition in soils along an elevational transect

Better understanding of the ecology of rhizobia in relation to its host and its environment may aid in making appropriate management decisions for a wide range of conditions. The Maui Network of Experimental Sites (Maui Net), Hawaii, provides us with a field laboratory of various edaphic and climatic conditions. The purpose of our investigation was to test selected genotypes of soybean in field environments that differed in soil and air temperatures for *B. japonicum* strain competition, nodulation, and early nitrogen accumulation. We controlled other variables by equalizing pH to 5.5-6.0, maintaining soil moisture at 3.0 x 10e4 Pa tension, and applying nutrients other than N at optimal levels.

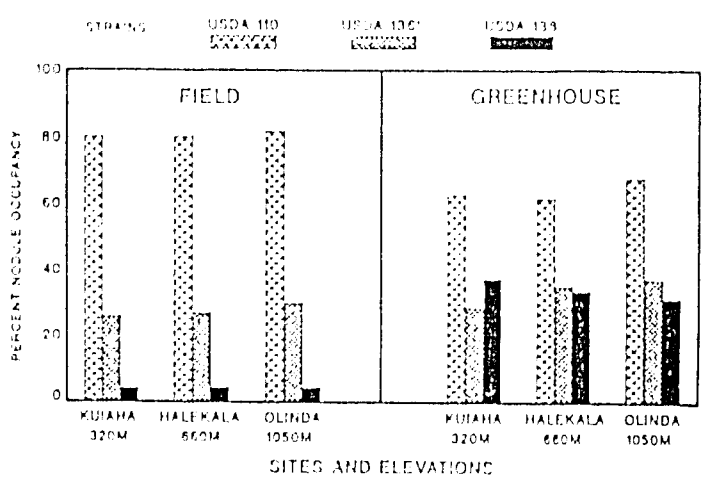
Three sites were selected along the slopes of the volcanic Haleakala mountain on the island of Maui at elevations of 320 M (Kuiaha), 660 M (Haleakala), and 1050 M (Olinda). The soils at these locations are classified as humoxic tropohumult (clayey, ferritic, isohypothermic), humoxic tropohumult (clayey, oxidic, hypothermic), and entic dystrandept (medial over loamyskeletal, isomesic). The mean soil/air temperatures during the 45 days of crop growth were 25.3/22.8, 22.9/20.9, and 20.7/18.2 degrees C at the respective elevations.

Five soybean varieties were planted at each site on the same day. These varieties and their respective maturity groups were Clay (00), Clark (IV), D68-0099 (VI), N77-4262 (VI), and Hardee (VIII). Seeds were inoculated before with a peat inoculum containing a mixture of equal numbers *Bradyrhizobium japonicum* strains, USDA 110, USDA 136b (CB1809), and USDA 138. Nodule occupancy of these serologically distinct strains was determined by fluorescent antibody technique.

These varieties were also planted in pots containing similarly amended soils from the three sites in a greenhouse experiment at the NifTAL headquarters. Inoculation was identical to field procedures.

The competition patterns between the three B. japonicum strains were generally independent of host, soil type or soil temperature, indicating that the strains have inherent differences in competitive ability (Figure 1). Strain USDA 110 was the best competitor, occupying on the average 81 and 64% of the nodules in the field and greenhouse experiments respectively. Strain USDA 138 was the least successful

Figure 1. Proportion of Nodules Formed by *B. japonicum* Strains on Soybean Grown on Three Soils



in the field (4%), although it formed 34% of the nodules in the greenhouse. Variety Hardee, which carries the Rj2 gene and is incompatible with strain USDA 136b, formed the maximum number of nodules with USDA 110.

Nodule number and mass decreased with decreasing temperature for all varieties, with nodule mass being affected more than nodule number. These decreases were directly correlated with temperature effects on plant yields. With the exception of Hardee, late maturing varieties tended to form more nodules than early maturing varieties; however, there was not a similar increase in nodule mass.

In summary, our experiments identified USDA 110 as the best competitor compared with USDA 136b and USDA 138 for nodule occupancy, regardless of large differences in soil type, soil temperature, or maturity group of soybean. Nodulation and plant growth at 45 days after planting were drastically reduced by a 5 degree C decline in both soil and air temperatures. Large differences in soybean plant growth between sites had little effect on competition between strains. None of the nodulation and plant growth parameters were related to occupancy of strains in the nodules. Our results indicate that rhizobial strains superior in competitiveness can be identified for a broad range of host genotypes, soil types, and temperatures.

- Performance of Field Grown Legumes at Varied Temperatures and Soil N Availability

To assess the production potential of legumes in specific geographical areas, a knowledge of the extent and nature of their adaptation to environmental limitations is necessary. An understanding of the processes of legume adaptation to temperature may be useful in identifying genotypes suited for varied tropical elevations.

The experiment was designed to evaluate the mechanisms of adaptation of soybean (*Glycine max*) and the common bean (*Phaseolus vulgaris*) to cool temperature under varying levels of N nutrition. The experiment was conducted at two sites (320 and 1050 m) in an elevational transect. The mean soil/air temperatures at the two sites were 25/23 and 21/19 C. The two legumes were grown inoculated at three levels (9, 120, 900 kg ha^{-1}) of N application. Sample harvests were made at stages full bloom/ beginning pod (R2/R3), beginning seed/ full seed (R5/R6), and at physiological maturity (R7).

Days to flowering and maturity were delayed for both soybean and the common bean at the 1050 m compared to the 320 m elevation (Table 3). The effect of cool temperature at the higher elevation on crop duration was more pronounced for soybean than the common bean. Increased accumulation of plant N delayed the maturity of soybean at both sites; the average days to R7 of soybean ranged from 96 days at the 9 kg N ha^{-1} to 102 days at the 900 kg N ha^{-1} application. The average dry matter accumulation by soybean and the common bean were similar across elevations (Table 4). N application increased the dry matter of both legumes. The maximum N response in dry matter of 41% over the 9 kg N ha^{-1} application was observed for soybean receiving 900 kg N ha^{-1} at the 320 m site. Total N accumulation followed similar trend as DMA, but significant response to N application above 120 kg N ha^{-1} was observed only at the 320 m site (Table 5). In spite of similar dry matter yield among legumes and elevations, soybean at the 320 m elevation accumulated the maximum average nitrogen of 271 kg ha^{-1} by maturity which was on average 48% higher than the rest. Response to N application was maximum for soybean and at the 320 m site. The rates of both

dry matter and nitrogen were affected adversely at the 1050 m site, but greater reduction occurred in the rates of nitrogen accumulation. However, the reduced rates of DMA and TNA at the higher elevation was offset in both legumes in varying degrees by prolonged growth duration and greater partitioning of dry matter into leaves. The leaf area index and the percent dry matter in leaf of soybean at the 1050 m site were always higher than at the 320 m site (Table 6). The harvest index of soybean at the 1050 m site was only 0.36 compared to 0.48 at the warm site, resulting in drastic reduction in grain yield.

The experiment demonstrated that the reduced rates of growth and N accumulation in cool environment may be offset by prolonged growth duration and greater partitioning of dry matter into leaves. However, equivalent total dry matter production by soybean among cool and warm sites may not result in similar seed yields. Both legumes respond to N application, but the response is modulated by the potential N requirement in the environment in relation to the native soil N. While growth of both soybean and the common bean may adapt to a cool environment, the latter exhibits greater tolerance in dry matter production, N uptake and seed yield. The common bean may be a better crop for the higher elevation tropics than soybean.

Table 3. Days to first flower (R1) and physiological maturity (R7) of soybean and the common bean grown inoculated at two elevations at three levels of N application

Nitrogen regimen (kg ha^{-1})	R1[1]		R7[2]	
	Soybean	Bean	Soybean	Bean
	days			
	320 m elevation			
9	28	35	79	73
120	28	35	84	74
900	28	35	84	75
	1050 m elevation			
9	43	48	114	97
120	43	48	119	98
900	43	48	120	98

[1] Days to first flower

[2] Days to physiological maturity

Table 4. Effect of elevation and N application levels on dry matter accumulation by soybean and the common bean grown inoculated in the field.

Nitrogen regimen (kg N ha^{-1})	Soybean	Common bean
	— 320 m elevation —	
9	5971	5635
120	6985	6210
900	8439	6608
	— 1050 m elevation —	
9	6628	6324
120	6939	6572
900	6944	7260

Analysis of variance

Source	df	Significance
Elevation (E)	1	NS[1]
Species (Sp)	1	NS
Nitrogen (N)	2	***
Sp x N	2	NS

[1] NS: not significant; ***: significant at 0.001 probability level.

Table 5. Effect of elevation and N application levels on N accumulation by soybean and the common bean grown inoculated in the field.

Nitrogen regimen (kg N ha^{-1})	Soybean	Common bean
	320 m elevation	
9	241	142
120	252	147
900	320	189
	1050 m elevation	
9	209	149
120	229	170
900	220	192

Analysis of variance

Source	df	Significance
Elevation (E)	1	NS[1]
Species (Sp)	1	**
E x Sp	1	NS
Nitrogen (N)	2	***
Elev. (E) x N	2	*

[1] NS: not significant; *, **, and ***: significant at 0.05, 0.01 and 0.001 probability levels

Table 6. Effect of elevation on the leaf area index and dry matter partitioning into leaf of soybean and the common bean.

Elevation (m)	Soybean			Common bean		
	R2/R3[1]	R5/R6[2]	R7[3]	R2/R3	R5/R6	R7
	— Leaf area index —					
320	2.3	3.9	1.8	3.7	3.6	1.4
1050	3.6	5.3	2.5	3.8	3.6	1.7
	— Leaf dry matter (%) —					
320	48	25	9	42	21	8
1050	52	37	16	49	24	8

Analysis of variance

Source	df	Significance
Leaf area index		
Elevation (E)	1	**[4]
E x Sp	1	*
Stage of harvest (H)	2	***
Sp x H	2	***
Leaf dry matter (%)		
Elevation (E)	1	***[4]
Species (Sp)	1	***
E x Sp	1	**
Stage of harvest (H)	2	***
E x H	2	**
Sp x H	2	***
E x Sp x H	4	***

[1] Full bloom/ beg. pod stage
[2] Beg. seed/ full seed stage
[3] Physiological maturity
[4] Significant at 0.05 (*), 0.01 (**), and 0.001 (***) probability

- Effect of Temperature on the Phenology of Soybeans Adapted to Various Latitudes

The environmental adaptation of legume genotypes depends in part on the durations of phenological phases as affected by temperature, photoperiod and other variables. The duration of vegetative phase and the time of flowering are important considerations in the selection of suitable genotypes for cropping sequences of specific geographical areas.

The effects of temperature and nitrogen nutrition on soybean phenology was studied at field sites with elevations of 320, 660, and 1050 m on the island of Maui, Hawaii. The mean maximum/minimum air temperatures at the three elevations during the experiment were 27/20, 25/17, and 23/14, respectively. Photoperiod decreased from 13 h at planting to 11 h at the last harvest. Four determinate nodulating soybean varieties representing the maturity groups Clay (00), Clark (IV), D68-0099 (VI) and Hardee (VIII), one indeterminate variety of group VI N77-4262 (VI), and their non-nodulating isolines were planted at each site on the same day.

Soybean maturity was delayed with decreasing temperature mainly due to extended vegetative growth periods averaging of 23% and 64% longer at the 660 m and 1050 m sites compared to the lowest site (Table 7). The effect of site (temperature) on the duration of the seed-fill phase was minimal. Within each site higher maturity groups had progressively longer vegetative phases presumably due to photoperiodic effects. The rate of seed dry matter accumulation (kg $ha^{-1}day^{-1}$) decreased with increasing elevation, but did not show any consistent trends among maturity groups. Non-nodulating isolines matured earlier than their nodulating counterparts due to nitrogen stress (Table 8).

Table 7. Days to first flower (R1) and physiological maturity (R7) of nodulating isolines of five soybean varieties grown at three elevations.

	First flower			Physiological maturity		
Variety	320 m[1]	660 m	1050 m	320 m	660 m	1050 m
	days					
Clay (00)[2]	27.7E	31.0E	39.7E	73.0E	77.7E	93.0E[3]
Clark (IV)	30.0D	35.0D	46.0D	87.0D	92.0D	107.0D
D68 (VI)	33.7C	41.3C	55.7C	99.0C	101.0C	112.0C
N77 (VI)	38.0B	49.0B	66.0B	94.0B	105.0B	128.0B
Hardee(VIII)	42.3A	54.3A	73.7A	106.0A	117.0A	133.3A
Mean	34.3c[3]	42.1b	56.2a	90.0c	98.5b	114.7a

[1] Elevation in meters.

[2] Maturity grouping.

[3] Means followed by the same upper case letter within a column or lower case letter within a row for each category are not significantly different (P = 0.05) by Duncan's multiple range test.

Table 8. Mean days to first flower and physiological maturity for nodulating (nod) and non-nodulating (non-nod) isolines of five soybean varieties grown at two elevations.[1]

	First flower (R1)		Physiological maturity (R7)	
Varieties	Nod	Non-nod	Nod	Non-nod
	days			
Clay (OO)2	32.8a[3]	32.6a	75.3a	70.5b
Clark (IV)	37.0a	36.9a	89.5a	85.2b
D68 (VI)	43.6b	46.6a	95.5a	90.5b
N77 (VI)	51.0b	52.4a	99.5a	95.0b
Hardee (VIII)	56.8b	62.1a	111.5a	106.3b

The results indicate that low temperature prolongs vegetative growth of soybean independent of photoperiodic effects, but has minimal effect on post-flowering development under similar N nutritional levels. The extension of vegetative period by low temperature may be an important factor affecting productivity of soybeans when grown at higher tropical elevations.

- Grain Yield and Nitrogen Fixation by Soybeans Grown at Three Elevations

The exploitation of the legume-*Rhizobium* symbiosis in varied environments requires an understanding of factors that may affect fixed and soil N assimilation. Both management and environmental variables affect the outcome of the symbiosis. Temperature affects both maturity and N requirement of the legume, and soil N availability can therefore directly influence nitrogen fixation and yield.

Five soybean varieties adapted to various latitudes (maturity groups 00, IV, VI, and VIII) and their respective non-nodulating isolines were planted on the same day at three sites along an elevational transect in Hawaii to study the effect of temperature on yield and nitrogen fixation. The mean soil/air temperatures during the experiment were 24/23, 23/20, and 20/18 at 320, 660, and 1050 m elevations respectively. The seeds were inoculated at planting with *Bradyrhizobium japonicum*. Plants were harvested at physiological maturity.

Average grain yield and N accumulation at the highest site was only 41% of that at the lowest site (Tables 9 & 10) due to low temperature. Early maturing varieties were relatively more affected by low temperature than were later varieties. Within each site, late maturing varieties produced greater yield and accumulated more N than the early varieties. Nitrogen fixation contributed 80%, 66%, and 97% to total plant N at the lowest, intermediate and highest sites, respectively (Table 11). Differences between sites in the contribution of N fixation were due to differences in the availability and uptake of soil N.

Table 9. Grain yield of five nodulating soybean varieties grown at three different elevations.

	Varieties					
Site, elev. (m)	Clay	Clark	D68-0099	N77-4262	Hardee	Mean
	00[1]	IV	VI	VI	VIII	
	kg ha^{-1}					
Kuiaha, (320)	3223	4424	4273	3530	4833	4057A[2]
Haleakala, (660)	2803	3066	3721	3526	3601	3344B
Olinda, (1050)	517	1357	1903	2580	1877	1647C
Mean	2181d[2]	2849c	3299ab	3212b	3437a	

[1] Indicates maturity group designation of the variety.
[2] Data followed by the same letter within the column or row are not significantly different (P = 0.05) by Duncan's multiple range test.
[3] Values in parentheses are stover yields.

Table 10. Total nitrogen accumulation by five nodulating soybean varieties grown at three different elevations.

	Varieties					
Site, elev. (m)	Clay	Clark	D68-0099	N77-4262	Hardee	Mean
	00[1]	IV	VI	VI	VIII	
	kg ha^{-1}					
Kuiaha, (320)	236	317	316	260	349	295A[2]
Haleakala, (660)	199	246	271	258	276	250B
Olinda, (1050)	44	99	128	186	144	120C
Mean	160d[2]	221c	238b	234bc	256a	

[1] Indicates maturity group designation of the variety.
[2] Data followed by the same letter within the column or row are not significantly different (P = 0.05) by Duncan's multiple range test.

Although total N assimilation between varieties differed in some cases by more than 400%, the proportions of total nitrogen derived from fixation were similar within a site. On a per-day basis, N accumulation was similar among varieties at a site. Therefore the relative contribution of nitrogen fixation to total plant nitrogen was yield independent.

Since the proportion of N derived from N_2 fixation is yield independent at a site, it may be possible to predict the relative contributions of soil N and symbiotic N_2 fixation to soybean through characterization of the processes that affect N availability at a site. The results also indicate that soybeans can be successfully grown at different elevations in the tropics with symbiotic nitrogen fixation as the primary source of plant nitrogen.

Table 11. Percent of total nitrogen derived from symbiotic fixation by five soybean varieties grown at three elevations.

	Varieties					
Site, elev. (m)	Clay	Clark	D68-0099	N77-4262	Hardee	Mean
	00[1]	IV	VI	VI	VIII	
	%					
Kuiaha, (320)	85	82	85	70	80	80B[2]
Haleakala, (660)	62	71	65	66	68	66C
Olinda, (1050)	95	98	98	98	96	97A
Mean	81ab[2]	84a	83a	78b	82ab	

[1] Indicates maturity group designation of the variety.

[2] Data followed by the same letter within the column or row are not significantly different (P = 0.05) by Duncan's multiple range test.

- The Efficiency of NO_3 Assimilation is One Mechanism Which Determines N_2 Fixation by Legumes

The availability of soil N is a major criteria of the fertility of soils. While mineral N is a necessary input for non-legume crops it can be considered a stress upon the legume symbiotic system. Legumes provided mineral N produce normal and sometimes even greater yields than legumes relying solely on BNF for their N source but increasing mineral N availability reduces the amount of N_2 fixed by the crop.

Numerous inoculation trials at NifTAL and other institutions have indicated that *Phaseolus vulgaris* does not respond to inoculation as frequently as soybean even when there are no native *Rhizobium phaseoli* present in the soil. Part of this phenomenon may be due to the lower yield potential and N requirements of *Phaseolus* compared to soybean. Present field experiments at NifTAL have indicated that *Phaseolus* also has a much higher N fertilizer use efficiency (or measurement by ^{15}N tracer methods) than soybean in early growth. It is during early vegetative growth that the symbiosis is being established and may be the affected by mineral N. We chose *Phaseolus* and soybean as comparative systems to evaluate physiological mechanisms that condition NO_3 assimilation and N_2 fixation.

Experiments were conducted in large solution culture tanks (1260 L capacity each) that permitted us to buffer solution NO_3 at concentrations equivalent the soil solution. *Phaseolus* and soybean were planted in tanks with 0.00, 0.05, 0.10, 0.25, and 1.5 mM NO_3 as KNO_3. Potassium concentrations were made equal between treatments by the addition of K_2SO_4. All solutions were enriched with ^{15}N so N_2 fixation could be measured. N concentrations were adjusted daily and measured values remained within 10% of target concentrations. Plants were harvested at 33 DAP.

The growth rate of *Phaseolus* at 0.0 mM N was equivalent to soybean but a magnitude higher at .05 mM N. The growth response of *Phaseolus* to mineral N reached a plateau at 0.05 mM N while shoot weight continued to increase with each increase in N concentration (data not shown). The 0.05 mM treatment reduced nodule weight of *Phaseolus* to 18% of the control while soybean nodule weight remained at 70% of the control (Table 12) and was many times greater than *Phaseolus*. Leaf nitrate reductase activity (there was no root activity in either species) followed a similar pattern as shoot weight and nodulation (Table 13). Root length per unit root dry weight increased with the availability of NO_3 (Figure 2). *Phaseolus* had significantly greater root length than soybean at every level of NO_3. Analysis of ^{15}N isotope ratios and N content of plants remains to be finished in order to calculate actual N_2 fixation in the system.

Table 12. Nodule dry weight of soybean and *Phaseolus* bean grown at five concentrations of NO_3 in solution.

NO_3	Soybean	Phaseolus
mM	..... mg plant^{-1}	
0.00	164	216
0.05	114	39
0.10	74	7
0.25	50	17
1.50	8	3

Source of Variation

	d.f.	F
NO_3	4	16.6***
Species	1	2.6 NS
NO_3 x Species	4	2.1 NS

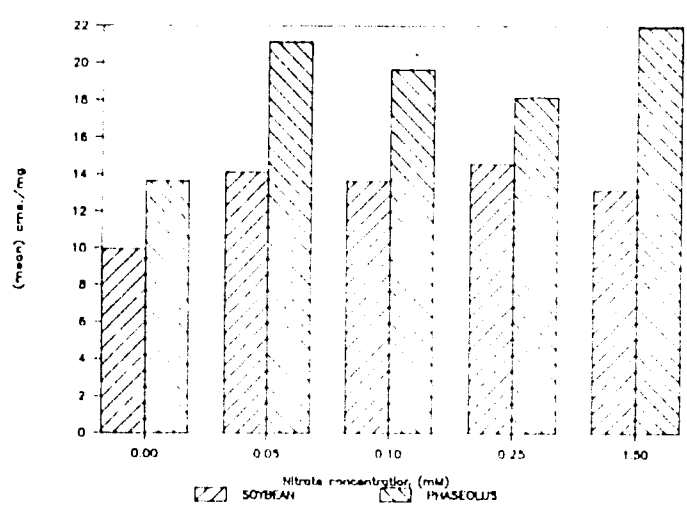
Table 13. Leaf nitrate reductase activity of soybean and *Phaseolus* bean grown at five concentrations of NO_3 in solution.

NO_3	Soybean	*Phaseolus*
mM	... g NO_2 g^{-1} fresh wt h^{-1} ...	
0.00	54	94
0.05	108	172
0.10	184	192
0.25	250	188
1.50	280	192

Source of Variation

	d.f.	F
NO_3	4	48.6***
Species	1	0.8 NS
NO_3 x Species	4	12.0***

Figure 2. Comparative Root Lengths of 2 Legumes at Different Nitrate Levels



These results indicate that *Phaseolus* despite greater growth and demand for N assimilation in early development relies to a greater extent on mineral N sources than soybean. One mechanism involved in this phenomenon is root morphology and nitrate reductase enzyme activity in the plants. The fine root structure of *Phaseolus* provides greater surface area for NO_3 assimilation with a reduced carbon cost to the plant. Whether greater nitrate reductase activity at low NO_3 concentrations is due entirely to the greater rate of NO_3 assimilation by roots, or indicates a greater efficiency in enzyme activity cannot be determined in this study. Understanding the mechanisms that result in efficient NO_3 assimilation will help to develop strategies which will enhance N_2 fixation by reducing host reliance on soil N resources. Maintenance of crop yield with reduced loss of soil N reserves will ultimately enhance productivity of crop systems with less reliance on fertilizer inputs. Selection of legume for particular soil environments could be undertaken with the goal of conserving soil N. We are now implementing experiments which will elaborate on mechanisms which determine NO_3 assimilation and hence affect the amount of N_2 fixed by crop species.

Development of environmentally based models to predict the growth, persistence, and competitiveness of rhizobia

Edaphic and biotic soil properties affect the expression of symbiotic activity. Soils, conditioned by climate and vegetation differ considerably in the symbiotic effectiveness of their native populations. While native populations of microsymbionts may be in balance with the nutrient requirements of natural plant communities, the introduction of new plant species or increasing the nutrient requirement of the plant community affects this balance. Crop management for high yield often raises the demand for nutrients from symbiotic sources beyond the supply capability of the native population. Figure 3a demonstrates this concept. Legume species grown in these field trials were managed to attain maximum yield. The relative response to legume inoculation with rhizobia was site specific and a function of native populations of rhizobia.

Edaphic factors such as availability of the primary nutrient supplied by the symbiosis plays a role in the expression of the symbiosis. Figure 3b demonstrates how the proportion of plant N derived from N-fixation affected the response to inoculation. Greater soil N availability (lower proportion of plant N derived from fixation) reduced the need for symbiotically derived N and, therefore, the introduction of exotic rhizobia has less effect on plant yield.

One requisite to optimizing the nitrogen fixing system is to be able to manage microsymbiont populations. This requires non-site-specific models of rhizobial population dynamics. At present NifTAL, with support form NSF and USAID, is generating a comprehensive database from 27 sites (18 MauiNet sites and 19 sites in developing nations) representing a range of ecological conditions.

Data on the plant community, i.e., plant species diversity, density and above and below ground biomass, climate, and soils including enumeration of rhizobial populations is being gathered. Regression and multivariate models are being developed that quantify the relationship between the target rhizobia and abiotic and biotic environmental influences.

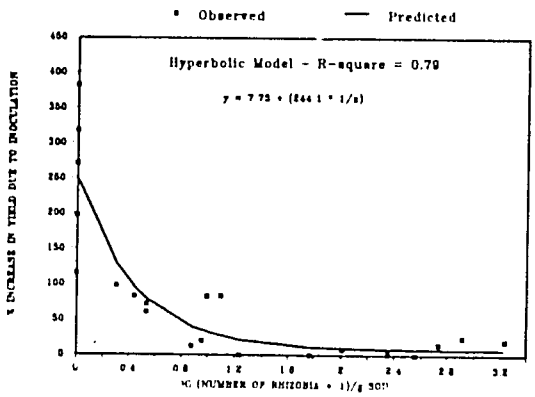


Figure 3a. Effect of Native Populations on Legume Response to *Rhizobium* Inoculation in The MauiNet. The native organisms may also be present in sufficient quantities and effectiveness to meet the nutrient requirments of the plant community. This figure demonstrates the relationship between native rhizobial populations and plant response to the introduction of exotic rhizobia. The variable plant response to inoculation when there are no native rhizobia is due to climatic effects and plant yield potential and edaphic factors at the different sites. Data are the result of field trials at seven MauiNet sites.

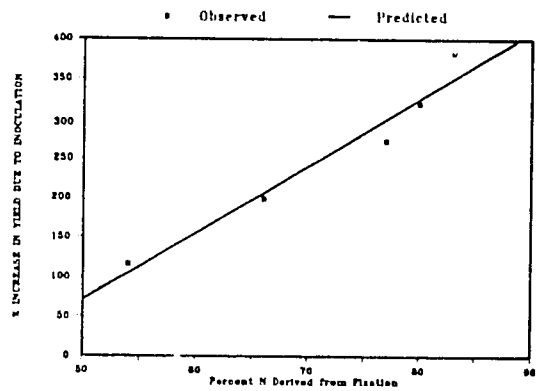


Figure 3b. The Relationship Between the Proportion of Plant N Derived From N-fixation on Legume Response to Inoculation. This figure shows the relationship between the proportion of plant nitrogen that is either symbiotically produced or soil-N derived and the response to introducing rhizobia. Greater symbiotic activity due to edaphic factors such as low soil organic matter and nitrogen availability is directly related to the relative response to inoculation. These results demonstrate the complex interactions which must be understood to fully exploit symbiosis.

RHIZOBIUM ECOLOGY

INTRODUCTION

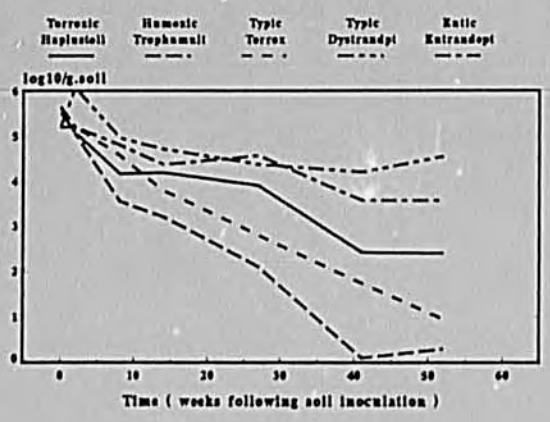
The nature of microsymbiotic population in terms of number, symbiotic potential and ecological adaptation affects the establishment and expression of the symbiosis and therefore has important implications in our ability to fully benefit from and manipulate symbioses in both managed and natural ecosystems. Using a number of MauiNet sites (Table 15) we have begun to elucidate which biotic and abiotic factors determine the nature of indigenous rhizobial populations and control the fate of rhizobial species introduced into new environments.

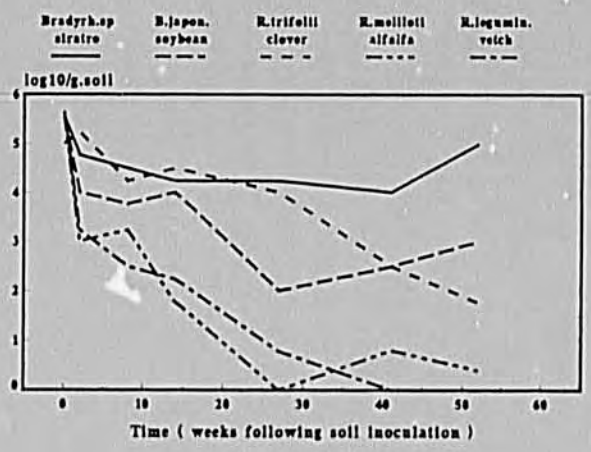
Ecological interactions were found to play a major role in determining the nature, extent, and diversity of native *Rhizobium* and *Bradyrhizobium* species (Table 16). Furthermore, the environment markedly affected the establishment and survival of introduced rhizobia (Figure 4a and 4b). Using this and other data presently being gathered, first generation predictive models of rhizobial population dynamics are being formulated. Testing and refinement of the models is needed.

Table 15. Location, Mean Annual Rainfall (MAR), Elevation, Soil Description, and Vegetation Formation of Some MauiNet Sites.

Site	MAR[a] (mm)	Soil[b] (great-group)	pH	Organic C (%)	Elevation (m)	Legume cover (%)	Vegetation formation[c]
Hashimoto Farm	322	Haplustolls	6.8	1.1	37	6.5	Short grass savanna (Cenchrus ciliaris) with isolated deciduous shrubs (Leucaena leucocephala) and trees (Prosopis pallida)
Sugar Waiakoa	358	"	7.9	1.6	187	3.0	Short grass savanna (C. ciliaris) with isolated deciduous trees (P. pallida)
Sugar Maalaea	372	"	7.4	1.2	38	2.0	Ephemeral short mixed grassland with forbs (Crotolaria sp.)
Kula Agricultural Park	375	"	7.5	1.5	366	4.7	Short grass savanna (C. ciliaris) with isolated deciduous shrubs (L. leucocephala)
Pasture Waiakoa	380	"	7.0	2.0	366	1.0	Short grass savanna (C. ciliaris) with isolated deciduous trees (P. pallida) and cacti
Sugar Paia	495	Torrox	6.8	1.5	61	4.5	Short grass savanna (Chloris gayana) with shrubs (L. leucocephala)
Pulehu Station	565	Haplustolls	6.1	4.0	640	36.7	Tall grass savanna (Panicum maximum) with forbs (Desmodium uncinatum; Vicia sativa), isolated shrubs (L. leucocephala) and trees (Acacia mearnsii)
Kula Station	845	Eutrandepts	6.8	8.7	945	22.1	Grassy pasture (Pennisetum clandestinum) with forbs (Trifolium repens, Medicago polymorpha, D. uncinatum and V. sativa)
Pasture Kekoa	1060	Dystrandepts	6.3	11.2	844	33.0	Grassy pasture (P. clandestinum) with forbs (T. repens, M. polymorpha, D. uncinatum and V. sativa)
Pasture Puupahu	1100	"	6.1	14.5	1646	3.3	Mixed grass pasture with forbs (T. repens)
NifTAL Project	1130	Haplustolls	6.8	2.4	110	11.0	Mowed grass (Eremochloa ophiuroides) with forbs (Desmodium canum, Desmanthus virgatus and M. polymorpha)
Olinda Prison Farm	1200	Dystrandepts	5.2	7.5	1067	50.2	Grassy pasture (P. clandestinum) with forbs (T. repens and V. sativa)
Haleakala Station	1800	Tropohumults	5.3	3.6	640	39.5	Grassy pasture (P. clandestinum) with forbs (Desmodium intortum, T. repens)
Kuiaha Site	1875	"	4.9	2.9	287	26.8	Grassy pasture (Digitaria decumbens) with forbs (D. canam, Indigofera suffruticosa, Cassia leschanaultiana) and isolated trees (Acacia koa)

[a]From Median Rainfall, State of Hawaii (1982). State Department of Land and Natural Resources, Circular C88.
[b]From Soil Survey of the Islands of Kauai, Oahu, Maui, Molokai and Lanai (1972). USDA Soil Conservation Service.
[c]After D. Mueller-Dumbois and H. Ellenburg (1974). Aims and Methods of Vegetation Ecology, Appendix B. John Wiley and Sons, Publisher.





Figures 4a and 4b. Fate of Rhizobia Introduced into the Environment. Edaphic and climatic factors can affect survival of microsymbionts. These figures show how soil characteristics (soil great group) affect survival of different rhizobial species.

Plots within the MauiNet were inoculated at time 0 with 10^5 cells of three serologically distinct strains from each of six different rhizobial species. Plots were kept free of plants and rhizobial survival monitored for one year. Soil type has a large effect on survival of a single species (Figure 4a). Significant species differences are also evident in an individual soil (Figure 5b). Ultimately, these models may be useful in predicting the behavior of genetically altered organisms introduced into the environment (Woomer, Singleton, & Bohlool. In press).

Site	*R. trifolii*	*R. meliloti*	*R. leguminosarum*	*L. loti*	*B. japonicum* sp.	Total
Hashimoto Farm	nd[b]	nd	nd	2.18	1.65	2.29
Sugar Waiakoa	nd	nd	nd	2.60	0.57	2.61
Sugar Waalaea	nd	nd	nd	2.73	1.51	2.76
Kula Ag Park	nd	nd	nd	1.30	1.34	1.62
Pasture Waiakoa	nd	nd	nd	nd	1.11	1.11
Sugar Paia	nd	nd	nd	1.49	1.50	1.80
Pulehu Station	0.48	nd	3.21	1.20	3.03	3.43
Kula Station	3.74	3.47	3.90	nd	4.00	4.42
Pasture Kekaa	3.13	1.36	3.77	nd	3.90	4.18
Pasture Pagwhu	3.26	nd	nd	nd	nd	3.26
NiFTAL Project	nd	1.78	nd	1.91	2.14	2.48
Olinda Primeu Farm	3.77	nd	2.24	nd	1.78	3.78
Haleakala Station	3.46	nd	nd	nd	4.76	4.78
Kulaha Site	nd	nd	nd	nd	4.50	4.50

[a]Rhizobia counts $\log_{10}$ (g soil)$^{-1}$
[b]nd = none detected.

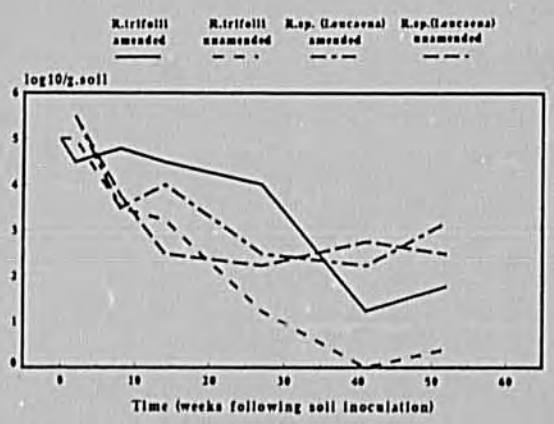


Table 16. Diversity of Native *Rhizobium spp.* and *Bradyrhizobium spp.* Populations at 14 MauiNet Sites. Estimates were obtained using the most probable number technique and the homologous legume species as trap plants. *Bradyrhizobium* sp. are widely distributed, *R. meliloti* is more restricted in its environmental range. Soil weathering, chemical properties, temperature, rainfall, and biotic factors all vary between sites and influence microsymbiont species composition (Woomer, et al, in press).

Figure 5. Effect of Perturbation on Rhizobial Survival. Three serologically distinct strains of *R. trifolii* and *Rhizobium* sp. were introduced into a tropohumult at 10^5 cells/g soil. Half the experimental plots were limed to a pH of 7.0. Liming enhanced survival of *R. trifolii*, but not *Rhizobium* sp. demonstrating that perturbations can have differential effects on microbial populations.

- Ecological Indicators of Native Rhizobia in Tropical Soils

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

- Microbial Ecology of Symbiotic Organisms in the Free-Living State

For a symbiosis to occur the microsymbiont must be able to survive for at least short periods of time and sometimes reproduce in a particular environment in the absence of its hosp plant. In natural ecosystems where symbiotic associations may occur only sporadically during succession, the microsymbiont may need to exist saprophytically for extended time intervals. Little is known about the population dynamics of soil microbes including microsymbionts. Biotic and abiotic factors that affect microbial diversity, numbers, survival, and persistence are poorly understood. The genetic and physiological bases for saprophytic competence have yet to be established. Basic information on the relationship between environment and microbial populations will be useful not only for managing the symbioses emphasized at the Center but will provide a conceptual framework for predicting the fate and, therefore safety of, genetically altered microorganisms introduced into the environment.

- Ecological perturbations and microsymbiont populations

Development of natural resources and intensification of agriculture continue to accelerate in order to meet the expectations of a rising world population. However, agricultural practices and utilization of natural ecosystems can alter ecological processes substantially. Anthropogenic and natural perturbations affect microbial populations in many ways. Some perturbations may limit primary production by decreasing symbiont populations or altering the balance between symbionts and other organisms (Figure 5). Other perturbations may actually enhance the functioning of symbiotic systems and the demand for microbe plant symbioses to sustain primary production. To date, the effects of important natural and man-made ecological perturbations on microbial populations is largely unknown. Symbiotic potential can be defined as biomass, economic yield and nutrient content that is the result from symbiotic activity.

The environmental factors contributing to *Rhizobium* spp. abundance was examined in Kikuyu grass pastures in upland Maui. The sites comprised a 12-mile elevation neutral transect ranging in mean annual rainfall (MAR) from 810 to 1800

mm; pH from 5.0 to 6.5; extractable bases from 2.7 to 54 m.e./ 100 g soil and represented 3 soil great-groups. Soil populations of *Rhizobium* and *Bradyrhizobium* sp. as measured by the plant infection technique, (1.78 to 3.78 $\log^{10}$ cells/g soil), correlated significantly with increased host legume presence but was not related to soil pH, extractable bases or MAR.

One significant component of the model describing native rhizobial populations in terms of ecological indicators, was the legume component of the site vegetation. To further define the role of the legume component, we examined three systems: 1) a *Vigna unguiculata/Glycine max* experimental field; 2) *Trifolium repens*/Kikuyu grass pastures; and 3) stands of *Desmodium intortum*. Presence of specific host legumes in cropping systems resulted in a 148-fold increase in native rhizobia. Nitrogen application did not enrich the native population. In pastures, increases of specific host legumes resulted in 29 to 16,000-fold increases of specific *Rhizobium* spp.

INOCULATION TECHNOLOGY

INTRODUCTION

Legume seed inoculation with high quality carrier-based inoculants containing effective and compatible strains of *Rhizobium* can increase the yield of legumes. One of the major constraints limiting maximal benefits from biological nitrogen fixation for farmers in the tropics is the availability of high quality inoculants and its performance under field conditions. We conducted several studies to examine the following: 1) the feasibility of multi-purpose inoculants and inoculants containing "helper" organisms; 2) the advantages of mixed vs. single-strain rhizobial inoculants; 3) variation of host physiological responses to low inoculum densities which may result from poor quality or poor survival of rhizobial inoculants; 4) host x strain interactions in *Phaseolus*; and 5) *Rhizobium* for leguminous trees.

- Multipurpose Inoculants and Helpers

The quality of inoculants can be affected by a variety of factors which directly or indirectly influence the microsymbiont when it is surviving in the carrier to the time when it is inoculated onto the seed. Among the biotic factors that influence survival and performance of rhizobia might be "helper" microorganisms such as *Lipomyces starkii* which produce oil which may protect rhizobia from dessication during storage and exposure to high temperatures. Some other bacteria, e.g., *Pseudomonas fluorescens* produce siderophores which may enhance rhizobial survival and help its iron nutrition. Fungal organisms, e.g., *Trichoderma* sp. can aid in suppressing root disease of newly sown legumes and improve legume stands. Also,the increasing use of *Bacillus thurigiensis* as a biological insecticide opens new approaches to increasing the spectrum of uses for the legume inoculant if rhizobia can co-exist with *B. thurigiensis*. Multipurpose and affordable legume inoculants offer tremendous potential for increasing crop yields through biological nitrogen fixation for the farmers in developing nations.

The farmer's practice of coating legume seeds with rhizobial inoculant prior to planting offers an opportunity to introduce other beneficial organisms or chemicals into the rhizosphere of the seedlings. The success of such strategies is dependent on the compatibility between the rhizobial strains and the chemicals or other organisms to be employed as co-inoculants. We conducted several studies to examine the potential of various multi-purpose inoculant systems.

Fungicides: The addition of fungicides at planting may be critical to the survival of germinating legume seeds. To determine the feasibility of multi-purpose inoculants which include fungicides, we conducted a plate-screening assay of 70 strains of *Rhizobium* and *Bradyrhizobium* strains against six commonly used fungicides. The rhizobial strains were from 31 leguminous species which were targeted for their economic importance in providing grain, forage, or fuelwood. We determined from our screening (Table 17) that the rhizobia were generally able to grow in the presence of the fungicides Benlate and PCNB; 2) the strains were generally sensitive to Thiram and Vitavax; and 3) there was strain-to-strain variability in the tolerance to Captan at the levels provided in the media. Our results indicate that rhizobial growth may not be affected by inoculation procedures which include concurrent applications of select fungicides.

Table 17. Fungicide* screening of *Rhizobium* strains.

	No. host species	No. strains	No. of tolerant strains					
			Ben.	Cap.	Man.	Thi.	Vit.	PCNB.
Rhizobium	9	22	22	2	1	1	3	22
Bradyrhizobium	22	48	46	25	2	1	2	48

*Fungicides and ppm active ingredient used in media.

Benlate	(DuPont "Benomyl")	500	Thiram	(DuPont "Tersan")	500
Captan	(Ortho "Rothocide")	200	Vitavax	(Uniroyal "Vitavax")	500
Manab	(Rohm Haas "Dithane")	200	PCNB	(Olin "Terrachlor")	500

Bacillus thuringiensis (Bt): We evaluated the growth and survival of *Rhizobium leguminosarum* bv. *phaseoli* TAL 182 str and a commercial strain of Bt as separate and mixed inoculants in a sterile peat carrier. Results in Table 18 show the Bt population increased ten fold and the *Rhizobium* three fold when the two organisms were introduced as combined dilute inocula into the peat. These increases were the same as those obtained when the organisms were incubated separately. After 16 weeks of storage, combining the organisms did not significantly affect their respective survival rates.

We established a small insectory for culturing the lepidopteran pest *Spodoptera mauritia* (army worm) for use of bioassays of the Bt toxin from organisms cultured in peat. Results of these studies were inconclusive because of the problems with the gradual loss of vigor and eventual population decline of the worms after 4-5 generations in captivity.

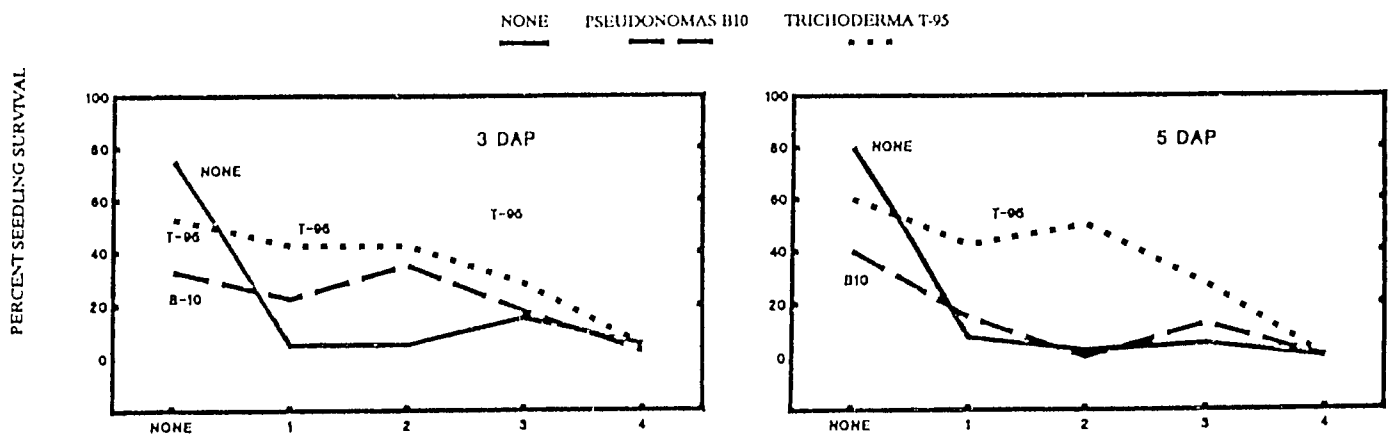
Table 18. Growth and survival of *Bacillus thuringiensis* and *Rhizobium phaseoli* TAL 182 str in separate and mixed peat inoculants during 16 weeks storage at 28 degree C

	bacteria g^{-1} inoculant at:			
Treatment	Time 0	2 weeks	8 weeks	16 weeks
182 only	$2.4x10^6$	$1.1x10^9$	$3.1x10^9$	$1.3x10^8$
Bt only	$2.2x10^6$	$4.5x10^7$	$4.5x10^7$	$3.4x10^7$
182 + Bt	$3.3x10^6$	$6.5x10^9$	$1.3x10^8$	$7.8x10^7$

Biocontrol agents: Poor legume stands are often due to seedling mortality caused by damping-off organisms like *Rhizoctonia solani* and various *Pythium* species. We tested two organisms which were isolated from soils naturally suppressive to damping-off diseases, for their ability to enhance seedling stands of *Medicago sativa* (alfalfa) and *Lens culinaris* (lentil). We used small pots containing artificial media infested with four levels of damping-off organism *Pythium myriotylum*. The antagonists, rhizosphere competent *Trichoderma harzianum* strain T-95 and siderophore-producing *Pseudomonas fluorescens* strain B10, had no effect on the survival of alfalfa in this system.

The presence of the antagonists improved lentil seedling emergence at low to moderate levels of *Pythium* infestation; however, by 5 days after planting (DAP) inoculation with the *Pseudomonas* strain did not overcome mortality due to post-emergence damping-off (Figure 6). Inoculation with *Trichoderma* resulted in increased survival of the lentil seedlings at low and moderate levels of *Pythium* infestation. When the disease organism was not present, inoculation with the antagonists resulted in lower germination rates compared to controls.

Figure 6. Lentil Seedling Emergency in Pythium Infested Media Effect of Biological Organisms



We screened 35 strains of *Rhizobium* and *Bradyrhizobium* for their compatability with the *Pseudomonas* strain (Table 19). Antibiosis effects were evaluated by cross-streaking the strains on 6 media. Rhizobial growth was generally inhibited by the *Pseudomonas* strain when the media lacked iron. When peat extract was incorporated into the media, all of the rhizobial strains grew well. These results indicate that rhizobia and the siderophore-producer may be compatable in the peat environment.

Table 19. Number of rhizobial strains able to grow in the presence of siderophore-producing *Pseudomonas* strain B-10.

Rhizobia	No. strains	No. Strains growing on:					
		KB1	KB1 + Fe	KB2	KB2 + Fe	Peat	Peat + Fe
Rhizobium	21	7	17	10	18	21	21
Bradyrhizobium	14	0	13	0	11	14	14

KB1 = King's medium with proteose peptone at 10 g/l; KB2 = 5g/l. Fe = 200 ppm.

"Helper" organisms: We studied the yeast *Lipomyces starkii* because its oil producing ability may assist rhizobia in peat survive the effects of heat and dessication encountered during storage and transport. We cultured the yeast in a variety of media under aerated and static conditions. We obtained maximal growth and oil production using an aerated fermenter unit containing 10% crushed sugar cane extract.

The *Lipomyces* and *Bradyrhizobium* sp. CB756 grew two and three logs respectively in two weeks when cultures diluted in water were used to inoculate sterile peat. These growth rates were the same whether the organisms were combined or incubated separately in the peat. Counts after two weeks storage at elevated temperatures (37 and 42 degree C) showed that the yeast did not help the rhizobia survive adverse storage conditions. While oil produced by the yeast may prove to benefit the rhizobia, under the conditions of the study, oil production in the peat was low, and there were no mechanisms for release of the oil from the yeast cells into the peat environment.

Cost effective inoculant production systems: Extensive fermentation facilities are required for the production large quantities of broth culture for inoculation of non-sterile carrier material. While the cultures can be diluted 1:1000 in water to inoculate sterile peat, this cost-effective dilution method is not suitable for non-sterile carriers since competition from indigenous organisms affects the growth and enumeration of the dilute inocula. We studied the potential for using fungicidal and bactericidal materials which would inhibit the growth of the indigenous organisms in non-sterile carriers, yet allow the growth of rhizobia from dilute inocula.

Rhizobia were screened for their tolerance to fungicides (see Table 17) and compatible combinations were established. Streptomycin resistant mutants were developed for one *R. phaseoli*, one *R. loti*, and four strains *B. japonicum*. The antibiotic streptomycin was selected to control the growth of the indigenous microorganisms in the carrier because of its low cost and availability in agricultural formulations.

The ability of the carrier material to adsorb the antibiotic was evaluated by bioassays of extracts from the carriers amended with various levels of the streptomycin. Three materials, ground Canadian spaghnum peat moss, Nitragin Co. peat, and coal from India all showed a remarkable capacity to bind the streptomycin, remove it from the solution in the carriers' micro-environments, and thus render it ineffective against micro-organisms. Of the three carriers tested, the Indian coal was the most promising for use with the proposed production system. Approximately 60% of the streptomycin introduced into the coal was present in the extract, compared to 30% and 20% for the two peat carriers.

Single-strain vs multistrain inoculants

We previously conducted a pot experiment designed to investigate the merits and demerits of using single-strain versus multistrain inoculants in soil containing moderate levels of N. This experiment was repeated using soil that was amended with sugar cane bagasse to immobilize plant available soil nitrogen, and to magnify the effects of inoculation.

In chickpea (Table 20) no significant differences were seen in inoculation responses due to single or multistrains when plants were grown in soil where the plant available soil N was not immobilized. In contrast, in the same soil where plant available soil N was immobilized by the addition of sugar cane bagasse, the inoculation responses were magnified. The multistrain inoculation performed poorly as seen in the shoot weight and shoot nitrogen. Also, individual strain effectiveness were different. Inoculation response of the common bean (Table 21) followed a similar trend as seen with chickpea in that multistrain inoculation resulted in lower shoot N. Also, strain differences were significant when soil N was immobilized.

Table 20. Effects of single-strain versus multistrain inoculation of chickpea in unamended and bagasse amended soil.

	Unamended Soil			Soil + Bagasse		
Inoculation	Shoot dry wt(g pot^{-1})	Nodule dry wt(g pot^{-1})	Shoot N (mg pot^{-1})	Shoot dry wt(g pot^{-1})	Nodule dry wt(g pot^{-1})	Shoot N (mg pot^{-1})
TAL 480	2.62	0.1	55.7	1.23	0.31	23.3
TAL 620	2.50	0.06	52.2	1.61	0.30	27.0
TAL 1148	2.52	0.07	52.5	2.02	0.38	35.2
TAL 480 TAL 620 ** TAL 1148	2.56	0.07	52.5	1.42	0.35	25.4
Uninoculated*	2.37	0.00	48.2	0.17	0.00	1.0
LSD (0.05)	NS	NS	NS	0.24	NS	4.7

* Controls not included in analysis.

** Applied as a mixture

Table 21. Effects of single-strain versus multistrain inoculation of common bean in unamended and bagasse amended soil.

	Unamended Soil			Soil + Bagasse		
Inoculation	Shoot dry wt(g pot^{-1})	Nodule dry wt(g pot^{-1})	Shoot N (mg pot^{-1})	Shoot dry wt(g pot^{-1})	Nodule dry wt(g pot^{-1})	Shoot N (mg pot^{-1})
TAL 182	3.37	0.13	54.1	1.86	0.27	46.0
TAL 1383	3.39	0.06	44.2	0.95	0.11	14.8
TAL 1797	4.26	0.05	44.3	1.09	0.10	17.7
TAL 182 TAL 1383 TAL 1797 **	3.36	0.15	55.5	1.46	0.21	32.4
Uninoculated*	3.19	0.05	46.1	0.95	0.06	9.08
LSD (0.05)	NS	0.04	4.28	0.05	NS	13.6

* Controls not included in analysis.

** Applied as a mixture

In contrast to chickpea and common bean, multistrain inoculation did not affect shoot N in the soybean when soil N was immobilized (Table 22).

Table 22. Effects of single-strain versus multistrain inoculation of soybean in unamended and bagasse amended soil.

	Unamended Soil			Soil + Bagasse		
Inoculation	Shoot dry wt(g pot^{-1})	Nodule dry wt(g pot^{-1})	Shoot N (mg pot^{-1})	Shoot dry wt(g pot^{-1})	Nodule dry wt(g pot^{-1})	Shoot N (mg pot^{-1})
TAL 102	6.97	0.32	142.3	2.80	0.26	76.83
TAL 377	6.96	0.43	135.1	2.27	0.30	63.61
TAL 379	6.46	0.32	115.2	2.23	0.25	62.32
TAL 102 TAL 377 TAL 379 **	7.1	0.33	136.8	2.69	0.26	72.51
Uninoculated*	6.18	0.00	57.8	0.64	0.00	3.98
LSD (0.05)	NS	NS	15.1	0.25	NS	8.46

* Controls not included in analysis.

** Applied as a mixture

Our results indicate that the inoculation response with single-strain or multistrain inoculants may be governed by the plant availability or unavailability of soil N. Plant availability of soil N appears to be a significant factor in realizing inoculation response. Also, inoculation response to

single or multistrain inoculation may be site specific. More experimentation under field conditions are needed to verify the usage of single and multistrain inoculants.

- Host x Strain Interactions

A strain-cultivar interaction and strain competition experiment has been conducted on *Phaseolus vulgaris*. The data showed no significant differences in N-ase activity, nodule dry weights and shoot dry weights due to the strains used. However, all three parameters showed significant differences (p 0.001) due to cultivars. Strain-cultivar were also highly significant (p 0.001) but they were observed in the shoot dry weights only. Competition patterns for nodule occupancy by the two strains of *R. phaseoli* (TAL 187, TAL 1383) were cultivar specific. Results indicate that strain cultivar interactions must be considered when making strain recommendations.

We also conducted an experiment to investigate rhizobial specificity and N_2-fixation by hybrids of *Phaseolus acutifolius* x *P. vulgaris*. *P. acutifolius* and *P. vulgaris* are nodulated by *Bradyrhizobium* sp. and *Rhizobium phaseoli* .espectively. Results of the investigation showed that the hybrids could be nodulated effectively by only *R. phaseoli* or *Bradyrhizobium* sp. Highly significant host x *Rhizobium* interaction ($p<0.001$) was evident in acetylene reduction activity, shoot and nodule dry weights. Two hybrids identified as having high N_2-fixing potential were with *R. leguminosarum* biovar *phaseoli* in an Oxisol which contained a high background of native *Bradyrhizobium* population. It was concluded that inoculation of these hybrids with the correct inoculant is an important practice for planting in tropical soils.

- Rhizobium for Leguminous Trees

Trials to identify and evaluate *Rhizobium* for 10 species of leguminous trees being widely used in development projects has been initiated. Reforestation and tree planting as an integral part of agriculture activities are likely to involve planting trees in less than optimal conditions. Inoculating leguminous trees with rhizobia at the nursery stage is not only extremely economical but also may help cure one stress, low N availability, commonly experienced by seedlings in outplanting sites.

- Plant and Nodulation Response to Varied Inoculation Densities

Soil type, environment and crop system determine the numbers of rhizobia in soil. Environmental conditions also determine how many rhizobia applied to seed as inoculant will survive to infect plant roots upon seed germination. Farmers, therefore, are planting legume seeds without a guarantee of the numbers of invasive rhizobia available to meet their crop's N requirement. Past research has indicated that physiological mechanisms under host control of at least one legume species can compensate for reduced numbers of nodules caused by low numbers of rhizobia in the soil. These host controlled mechanisms regulate development of individual nodules to maintain the balance between host photosynthetic capacity and nitrogen fixation capacity. The net result is that within a certain range of densities of soil rhizobia, N_2 fixation can be maintained at optimum levels.

Some legume species tend to respond to inoculation with greater frequency than others. NifTAL and G.K.V.K. University of Agricultural Sciences have developed an initial screening program to determine whether pulses popular in both India and North America compensate for low numbers of rhizobia. Seven species of legumes were selected and inoculated with different numbers of the appropriate strain of rhizobia. The experiment was conducted in 1 L pots filled with horticultural grade vermiculite. Planting holes were made to a depth of 3 cm and 5 ml of inoculant (YEM broth culture diluted in sterile water) was applied to each planting hole. Pregerminated seed were then placed in the hole and sterile water applied to ensure contact between root and media. Plants were harvested between 28-35 days depending on species.

Inoculum density affected nodulation in terms of nodule number, nodule weight and the weight of an individual nodule (Table 23a, b). Shoot weight and N (data not shown) followed a pattern similar to nodule weight. The data indicate that some species are more affected by low inoculum density than others. *Macrotyloma* and *Phaseolus* had large numbers of nodules when only 10 cells were applied compared to *Arachis* which was almost nonnodulated at the same level. Other species were intermediate in their response. Species such as *Glycine* and *Arachis* exhibit a strong physiological response to low numbers of nodules. The average weight of a nodule on these two species increased more than 500% as the inoculum level was reduced from 10^6 to 10^1 cells/plant. *Glycine max* had the same total nodule weight at the 10^3 inoculum rate as the 10^6 rate even though nodule number was less than 50% of the 10^6 treatment. This physiological response is a mechanism which compensates for low numbers of rhizobia. This mechanism appears to be less effective in other species and should be examined further as an approach to ensuring adequate N_2 fixation by the crop under conditions with varied numbers of rhizobia available to the host.

Table 23a. Nodule number, nodule weight and average nodule weight of legumes inoculated with different numbers of rhizobia.

Inoculum density	*Cajanus cajan*			*Glycine max*			*Vigna unguiculata*		
	no.	mg	mg/nodule	no.	mg	mg/nodule	no.	mg	mg/nodule
10^1	9	11	1.3	2	29	14.5	59	254	4.3
10^3	32	40	6.0	57	528	9.3	201	826	4.1
10^6	128	188	2.5	127	508	4.0	319	1007	3.2

Table 23b. Nodule number, nodule weight and average nodule weight of legumes inoculated with different numbers of rhizobia.

Inoculum Density	*Macroxyloma uniflorum*			*Cicer arietinum*			*Arachis hypogaea*			*Phaseolus vulgaris*		
	no.	mg	mg/nodule	no.	mg	mg/nodule		mg	mg/nodule	no.	mg	mg/nodule
10^1	87	72	0.8	6	86	13	1	10	10.0	219	230	1.1
10^3	83	112	1.3	12	154	15	27	149	5.5	270	390	1.5
10^6	95	137	1.4	11	137	13	273	617	2.3	245	340	1.4

APPENDIX I

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Cassman, K.G., Whitney, A.S., and R.L. Fox. 1981. Phosphorus Requirements of Soybean and Cowpea as

Affected by Mode of N Nutrition. Agron J 73:17-22.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Eriksen, F.I., and A.S. Whitney, 1984. Effects of Solar Radiation Regimes on Growth and 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.

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

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

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.

47

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 Asse. sment Workshop, Background Papers for Innovative Biological Technologies for Lesser Developed Coun'ries. Report Prepared for Committee on Foreign Affairs, U.S. House of Representatives. p. 243-273.

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

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

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, 1985. 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. Symbiosis. Crop Sci. 23:259-262.

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

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

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

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

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

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

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

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

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

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

Singleton, P.W., 1983. A Split-Root Growth System for Evaluating the Effect of Salinity on Components of the Soybean *Rhizobium japonicum*

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.

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

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

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

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

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

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

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

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

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

Somasegaran, P., H.J. Hoben, and V. Gurgun. Effects of Mixed Inoculants on the Competition for Nodulation and Nitrogen Fixation by *Rhizobium* Strains on Desi and Kabuli Chickpea Genotypes. Appl. Envir. Micro. In Press.

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

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

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

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

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

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

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

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

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

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

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

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

Woomer, P.L., 1979. Root Tuberization and Nitrogen Fixation by *Pachyrhizus erosus* (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.

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 an Growth of *Lablab purpureus* in Relation to *Rhizobium* Strain, Liming, and Phosphorus. Plant and Soil 53:329-339.

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

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

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

APPENDIX II

Abstracts of Work Published in 1987

Bohlool. Ben. Fluorescence methods for study of *Rhizobium* culture and soils. In. G.H. Elkan (ed) Symbiotic Nitrogen Fixation Technology, Marcel Dekker Inc., New York.

Direct examination of small portions of the natural environment under the microscope reveals interesting relationships between microorganisms, plants, and the physical environment. Conventional staining procedures can aid in improving the clarity of microscopic observations, but it is often difficult to distinguish microbial cells and filaments from organic and inorganic particles and fibers. Certain fluorescent dyes that selectively stain components of biological origin can be used to stain microbial cells and distinguish them from the environmental background. Dyes such as acridine orange and fluorescein isothiocyanate, which react with nucleic acids and proteins, respectively, have been used to stain and enumerate microorganisms directly in the natural habitat (see Parkinson et al., 1971; and Ramsey, 1978, for detailed references).

While fascinating physical relationships among different organisms can be discerned with such approaches, it is virtually impossible to identify the various components of microbial assemblage on the surface of azolla roots as revealed by acridine orange staining. But the small size and the nondescript morphology of the microbial partners impose serious limitations n the value of this type of approach.

Although microbes are, for the most part, nondescript in morphology, the do have unique chemical features that can be used to identify genera, species, and even strains of a particular microorganism. Serological techniques rely on differences in the chemical nature of particulate and soluble antigens of cells and provide a powerful tool for microbial differentiation. Immunofluorescence combines the specificity of immunological reactions with sensitivity and precision of fluorescence microscopy to facilitate simultaneous detection and identification of specific microorganisms in situ.

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

We studied the competition between *Rhizobium fredii* and *Bradyrhizobium japonicum* isolates for nodulation of soybean (Glycine max L. Merrill) cultivars Williams and Ai Jiao Zao grown in three different soils in pots. Two of the soils were from People's Republic of China, one from a soybean field in Honghu with no history of *Rhizobium* inoculation, and one from a rice field in Wuhan with no history of soybean cultivation. The Honghu soil contained *R. fredii* and *B. japonicum* (log total number g^{-1} = 5.82 0.58); whereas the Wuhan soil contained only *B. japonicum* (log total number g^{-1} = 2.80 0.52). Inoculation did not result in a significant increase in nodule number on plants in either soil. Uninoculated plants of both cultivars harbored only *R. fredii* in the Honghu soil and only *B. japonicum* in the Wuhan soil. Even when *B. japonicum* serogroups USDA 110 and USDA 136b (=CB 1809) occupied the majority of the nodules except when an isolate of *R. fredii* from the soybean soil was added in high numbers. In a Hawaiian soil devoid of *R. fredii* and *B. japonicum*, when

soybeans were inoculated with isolates of both species, most of the nodules were formed by *B. japonicum*. The *R. fredii* isolate could form up to 20% of nodules in this soil, but only on the Ai Jia Zao cultivar. In the Wuhan but not the Hawaiian soil, peat pelleting of seeds with equal numbers of two *B. japonicum* and one *R. fredii* isolates increased nodule occupancy by *B. japonicum* USDA 136b serogroup significantly as compared with when the same isolates were inoculated into the soil. The results reported here highlight the critical importance of being indigenous to the competitive success of *R. fredii* and *B. japonicum* in nodulation of their soybean host.

Thomas George, B. Ben Bohlool, and Paul Singleton. *Bradyrhizobium japonicum*-Environment Interactions: Nodulation and Interstrain Competition in Soils along an Elevational Transect. Appl Env Micro 53(5): 1113-1117.

The effects of temperature and soil type on interstrain competition of *Bradyrhizobium japonicum* and on nodulation and nitrogen accumulation in five soybean varieties belonging to four maturity groups were investigated at three sites devoid of soybean rhizobia along an elevational transect in Hawaii. Competition patterns of the three *B. japonicum* strains were unaffected by soil type or soil temperature. Strain USDA 110 was the best competitor, occupying on the average 81 and 64% of the nodules in the field and greenhouse experiments, respectively. Strain USDA 138 was the least successful in the field (4%), although it formed 34% of the nodules in the greenhouse. Nodule occupancy by *B. japonicum* strains was found to be related to soybean maturity group. Strain USDA 110 formed 61, 71, 88, 88, and 98% of the nodules in the field on Clay (00), Clark (IV), D68-0099 (VI), and Hardee (VIII), respectively. Strain USDA 136b formed few nodules on Hardee, an *Rj2* soybean variety incompatible with that strain, in both experiments. Nodule number and weight at the 1,050-m site were reduced to 41 and 27%, respectively, of those at the 320-m site because of the decrease in temperature. Nodule number increased with increasing maturity group number at each site; however, there was not a corresponding increase in nodule weight. Nitrogen accumulation decreased from 246 mg of N per plant at the lowest elevation site to 26 mg of N per pant at the highest elevation. While soil type and temperature had no effect on strain competition, temperature had a profound influence on nodule parameters and plant growth.

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

Soybean (*Glycine max* [L.] Merr. cv Davis) was grown in a split-root growth system designed to maintain control of the root atmosphere. Two experiments were conducted to examine how 80% Ar:20% O_2 (Ar:O_2) and air (Air) atmospheres affected N assimilation (NH_4NO_3 and N_2 fixation) and the partitioning of photosynthate to roots and nodules. Application of NH_4NO_3 to nonnodulated half-root systems enhanced root growth and root respiration at the site of application. A second experiment applied Ar:O_2 or air to the two sides of nodulated soybean half-root systems for 11 days in the following combinations: (a) Air to Both sides (Air/Air); (b) Air to one side, Ar:O_2 to the other (Air/Ar:O_2), and (c) Ar:O_2 to both sides (Ar:O_2/Ar:O_2). Results indicated that dry matter and current photosynthate ^{14}C) were selectively partitioned to nodules and roots where N_2 was available. Both root and nodule growth on the Air side of Air/Ar:O_2 plants was significantly greater than the AR:O_2 side. The

relative partitioning of carbon and current photosynthate between rots and nodules on a half-root system was also affected by N_2 availability. The Ar:O_2 sides partitioned relatively more current photosynthate than roots (57%) than nodules (43%), while N_2-fixing root systems partitioned 35 and 64% of the carbon to roots and nodules, respectively. The Ar:O_2 atmosphere decreased root and nodule respiration by 80% and nitrogenase activity by 85% compared to half-root systems in Air while specific nitrogenase activity of nodules in Ar:O_2 was 50% of nodules supplied Air. Results indicated that nitrogen assimilation, whether from N_2 fixation or inorganic sources, had a localized effect on root development. Nodule development accounted for the major decrease in total photosynthate partitioning to non-N_2-fixing nodules. Soybean compensates for ineffective nodulation by controlling the flux of carbon to ineffective nodules and their associated roots.

APPENDIX III

Abstracts of Meeting Papers and Posters Presented During 1987

Abaidoo, R.C., P. Somasegaran, and F. Kumaga. Host-*Rhizobium* Relationships and Nitrogen Fixation in *Voandzeia subterranea* (Bambarra groundnut).

Little is known on the host-*Rhizobium* relationships and nitrogen fixation in the bambarra groundnut. In an joint effort between the International Atomic Energy Agency (IAEA) and the NifTAL Project, 23 strains of rhizobia from 14 general of legumes, were evaluated on a Thai cultivar of *Voandzeia*. The symbiosis ranged from completely ineffective through moderate effectiveness to fully effective. *Bradyhrizobium* sp. (TAL 169 isolated from *Vigna unguiculata*) ranked most-effective while the wide-spectrum *Bradyrhizobium* strain CB756 was ineffective. In a host x *Rhizobium* interaction experiment with five host-genotypes and five *Bradyrhizobium* strains, the host-genotype significantly ($P > 0.001$) influenced shoot dry weight, total shoot N, nodule dry weight, and nitrogenase activity. The rhizobial strains were also significant sources of variation for shoot-dry weight (p 0.05), total shoot-N ($p > 0.001$), and nodule dry weight (p 0.01). There were no significant ($p > 0.05$) host x *Rhizobium* interactions indicating no specific combination was superior over the broad range of all treatments.

Caces, M. L. and B. B. Bohlool. Expression of Indigenous and Foreign Plasmids in *Rhizobium fredii*.

Five strains of *Rhizobium fredii* (HH 003, HH 102, HH 303, and type strain USDA 205) were examined for the presence of indigenous plasmids. To determine if symbiotic functions are controlled by genes on these plasmids, the strains were subjected to plasmid-curing treatments. the effect of a Sym plasmid from a heterologous species on *R. fredii* gene function was studied by the introduction of the *R. leguminosarum* Sym plasmid, JB5JI. The results showed that high molecular weight plasmids that are involved in determining symbiotic functions, as well as cryptic plasmids, are an integral part of the genetic make-up of *R. fredii*. In two of these strains, the loss of the ability to nodulate soybeans was correlated with the loss of a plasmid. The strains received and maintained pJB5JI but the pjB5JI genes were not expressed in the *R. fredii* genetic background. The introduction of the plasmid did not enable any of the *R. fredii* transconjugants to nodulate peas, nor did it restore the ability of the plasmid-cured transconjugants to nodulate soybeans. The *R. fredii* symbiotic genes were affected differently by the heterologous plasmid, with the transconjugants exhibiting different levels of symbiotic effectiveness that ranged from ineffective nodulation to N-fixation levels compared to those of the parental types. *R. fredii* gene expression in the presence of pJB5JI was unstable and changed with successive passage on the host plant.

George, T., P.W. Singleton, B.B. Bohlool, and D.P. Bartholomew,Development, Yield and Nitrogen Fixation of Soybean in the field as Affected by Temperature.

Five soybean varieties (maturity groups OO, V, VI, VIII) were planted with their non-nodulating isolines at three sites in Hawaii to study the effect of temperature differences due to elevation (320 m, 660 m, 1050 m) on growth,

development and nitrogen fixation. Average grain yield and nitrogen fixation decreased by 50% and 51% respectively, with a 5 degree C decline in average temperature form the lowest (24 degrees C) to the highest (19 degrees C) site. Mean days to flowering for the varieties increased 64% as temperature decreased from the lowest to the highest site. Days from flowering to maturity was unaffected by temperature. Late maturing varieties produced more grain and fixed more N than early varieties. Nitrogen fixed ranged from 42 kg for the 00 variety at the lowest site to 28 kg ha^{-1} for the VIII variety. The proportion of N derived from fixation which ranged from 62% to 98% did not vary between varieties within a site. Under constant photoperiodic regime, low temperature affected soybean development by increasing vegetative duration. The rate of N assimilation was relatively constant between varieties within a site despite large differences in total N assimilation. At low temperature, symbiotic N assimilation has a relative advantage over N uptake from soil.

H.J. Hoben, and P. Somasegaran. The NifTAL Training Program in *Rhizobium* Technology.

The NifTAL Project initiated a training program in *Rhizobium* technology to improve the technical skills of scientists in developing countries who were using rhizobia in research, development and production programs involving legumes. These courses are based on intensive practical exercises backed up by lectures. A manual has been produced to complement the instructions. It offers a detailed outline of the course itself which consists of 23 exercises contained in four sections: General *Rhizobium* Microbiology; Strain Recognition; Field and Greenhouse Experimentation; and Inoculant Production. The program is modular and allows sufficient flexibility to tailor appropriate course agenda to meet specific demands. For example NifTAL's two most recent courses emphasized inoculant production. Since 1976, NifTAL has conducted 14 courses through which 135 scientists from 47 countries have been trained. Future training efforts will focus on specialized areas in *Rhizobium* technology and will include modern methods for monitoring microorganisms in the environment, inoculant and other fermentation technologies, and genetic techniques.

Nakao, P., B. Bohlool, P. Singleton, and J. Hudson. Host Regulation of Nodule Development: Effect of Number of Primary Infections on Subsequent Development of Secondary Nodules in A Split-Root Growth System.

Previous research has demonstrated that prior inoculation of a soybean half-root system suppresses nodulation of the other side inoculated after a 7-day delay. We conducted two complementary split-root experiments on soybean to examine the effect of primary nodule number on secondary nodule development. In one case, we regulated the number of primary nodules by varying the density of the primary inoculant. Secondary nodule development was inversely proportional to primary inoculation level. In the second case, primary nodule number was dependent on the length of the primary roots available for infection at time of inoculation. Primary nodule number was thus regulated by inoculating at different times after root emergence into the root chamber. In this experiment suppression of secondary nodules was near complete (95-98%) in all treatments. These results demonstrate that the host has a specific nodulation potential for every growth condition. When primary inoculation is insufficient, the host will allow secondary nodule development to meet this potential. If inoculum is not limiting, the host will regulate nodule number according to growth conditions

and suppress secondary nodule development.

Pinchin, J.E., P.W. Singleton, and B.B. Bohlool. Models for Predicting Legume Response to *Rhizobium* Inoculation.

Inoculation of legumes with *Rhizobium* often does not result in increased yield. Soil physicochemical constraints, climatic and biological factors all condition plant response to inoculation. We have begun a three-year study to develop models to determine which environmental factors best predict where inoculation with *Rhizobium* will result in a plant growth response. *Glycine max, Phaseolus lunatus, Phaseolus vulgaris*, and *Vigna unguiculata* were grown under three N regimes (*Rhizobium* inoculation, 800 kg N/ha, and uninoculated) at three sites on Maui. Site soils differed in N availability and native numbers of *Rhizobium* capable of nodulating the four legumes ranged from 0 to 1700/g soil. Plants were harvested 25 days after planting and at harvest maturity. Plant response was measured by increased biomass, total shoot N, nodule number and mass, and final seed yield. Numbers of native *Rhizobium* were highly correlated with all response parameters in soils where N availability was low. Soils of moderate N indicate that the magnitude of the indigenous *Rhizobium* coupled with soil N availability in relation to host demand for N are of primary importance as predictors of inoculation response.

Singleton, P.W., P. Nakao, and B.Bohlool. Control of Nodule Development on Soybean Root Systems Growing Through Vertically Isolated Rooting Chambers.

A split-root growth system was developed that permitted soybean root systems to grow through wax seals separating two vertically stacked rooting chambers. The wax seals prevented movement of rhizobia between chambers. Experiments were conducted to examine the effect of plant age, root length, and placement of inoculum on the location and control of nodule development. When the top chamber was inoculated at planting and 7, 22, 47, and 53 hours (h) later, tap root nodules were the majority only at the first inoculation. Tap root nodule number was constant until the 47 h treatment. Lateral root nodulation increased with inoculation delay. Inoculation at 73, 125, 173 h when roots had penetrated the wax seal demonstrated that at a point in time, total nodule remained constant whether the top, bottom or both chambers were inoculated. Tap root nodules were observed in the top chamber when inoculation was performed four days after the tap root penetrated the wax seal. When soybean was planted in inoculated systems of two unequal lengths (1.5 cm top; 26.5 cm bottom), the top chamber had 31% of the nodule number in the bottom chamber. Results indicate that nodulation events within hours of germination exert significant control on the number and location of subsequent nodule development. After early growth, the root system of soybean has many more nodule sites available than are developed.

Somasegaran, P. and H.J. Hoben. Host-Rhizobium Specificity in *Phaseolus vulgaris* x *P. acutifolium* hybrids.

Phaseolus vulgaris (bean) and *P. acutifolium* (tepary) are nodulated by *Rhizobium leguminosarum* biovar *phaseoli* and *Bradyrhizobium* spp. respectively. A greenhouse experiment was set up to investigate the *Rhizobium* requirements and N-fixation traits in seven bean x tepary hybrids produce by Dr. L. Lewinson

(CIAT, Colombia). All hybrids were able to nodulate what both *R. leguminosarum* biovar *phaseoli* and *Bradyrhizobium* spp. but the effectiveness varied widely. Two hybrids which were nodulated by *R. leguminosarum* biovar *phaseoli* had shoot-dry weight and nitrogenase activity similar to that of the *P. vulgaris* parent. Main effects due to host genotype and *Rhizobium* were significant ($p < 0.001$) for all plant and N-fixation parameters. Also, significant ($p < 0.001$) host x *Rhizobium* interactions in all plant and N-fixation parameters indicated that the hybrids were specific in their *Rhizobium* requirements for effective symbiosis.

P. Woomer, P. W. Singleton, B.B. Bohlool. Models of Ecological Indicators of Native *Rhizobium* Populations on the Island of Maui, Hawaiian Islands.

Native *Rhizobium* populations limit response to inoculation of legumes. Populations of root-nodule bacteria were determined at 14 undisturbed sites on the island of Maui. Plant infections counts of *Rhizobium* for six legumes (*Trifolium repens, Medicago sativa, Glycine max, Leucaena leucocephala*, and *Macroptilium atropurpureum*) were conducted in the growth room. The sites ranged in mean annual rainfall form 320 to 2100 mm; elevation from 37 to 1650 m; and in soil pH from 4.6 to 7.9. Four different soil orders were represented in this study: Inceptisols, Mollisols, Ultisols, and an Oxisol. Total *Rhizobium* populations varied from 24 to 65,000 per g/soil. The most frequently occurring *Rhizobium* were slow growing cowpea-types present at 13 of 13 sites with a maximum of 58,000 per g/soil. *R. trifolii* and *R. leguminosarum* were present at higher elevations only. *R. meliloti*, when present, colonized soil in low numbers (40-60 per g/soil). The presence of a specific *Rhizobium* was well correlated with the occurrence of its appropriate host legume. Quantitative models are presented which describe the effects of legume hosts, soil climate, and soil chemistry on native *Rhizobium* populations.

Woomer. P. Reliability of the Plant Infection Technique among Hosts and Soils.

The plant infection technique, in which a code of positive (nodulated) and negative (non-nodulated) growth units is used to estimate populations, is widely used to enumerate soil *Rhizobium*. The internal a cohesion of the MPN code, the number of dilution steps from all positive to all negative growth units, is a useful criterion to assess the validity of experimental results when mean codes range is compared to the theoretical value. Six host legumes (*Trifolium repens, Medicago sativa, Glycine max, Leucaena leucocephala*, and *Macroptilium atropurpureum*) were examined in repeated plant infection counts of diverse Hawaiian soils. Only *V. sativa* did not differ significantly from the theoretical mean values. In declining order of precision: *V. sativa; G. max; T. repens; M. sativa; L. leucocephala; M. atropurpureum*. The 14 sites used in this study included 4 soil types: Haplustolls, Andepts, Tropohymults, and a Torrox. The effect of soil was not significant. There were significant host legume-soil interactions. *R. japonicum* and *R. meliloti* were poorly recovered from the Andepts, soils with amorphous clay fractions. Mechanisms which may account for the variable role of host and soil in recovery of rhizobia are presented.

The second Illustrated Concepts in Agricultural Biotechnology (ICAB) was published in 1987. It is titled "A Small Glass Fermentor for the Production of *Rhizobium* Inoculum" and authored by H. J. Hoben and P. Somasegaran.

APPENDIX IV

CONTRACTS AND GRANTS AWARDED OUTSIDE CORE FUNDING

Grant Title	Source	Amount
Soil Inoculation to Improve Crop Production	USAID/INDO-US	$190,000
Host Control of Nodule Formation & Development	USDA/Competitive Grants	80,000
Predicting Inoculation Response	NSF/Ecology	750,000
Effect of Some Stress Factors on the Nodulation Process	USDA	23,050
Production & Conservation of Fixed N in Tropical Cropping Systems: Interaction Between Management & Environment	USDA/USAID Limiting Factors	87,500
Quantifying, Characterizing & Maximizing BNF by Fast-Growing Nitrogen Fixing Trees	McIntire-Stennis/ USAID	80,277
Role of Nitrogen Fixation in Sustainable Agroforestry Systems	USDA/406	110,000

APPENDIX V

Visitors to the BNFRC in Bangkok, Thailand:

Jan.	12	Michael Green, BOSTID, Washington, D.C.
Jan.	7-30	R.J. Davis, NifTAL Project, Hawaii, USA
Jan.	14	Joel Cohen, USAID/TTI
		C.D. Crober, Canada Agriculture
		Peter Thomson, Pacific Seed Company, Thailand
		R. Kucey, Canada Agriculture
Jan.	16	Paul Bundic, ATI, Washington, D.C.
Feb.	23	John Slavics, ATI, Washington, D.C.
		Paul Bundic, ATI, Washington, D.C.
Feb.	27	Allen Hurdus, USAID, Bangladesh
		Doug Pickett, USAID, Burma
		Randy Cummings, USAID, Jordan
		Dennis Johnson, AID, Washington, D.C.
		Duane Acker, AID, Washington, D.C.
Mar.	2	John Slavics, ATI, Washington, D.C.
Mar.	9	John Thompson, NSW Dept. of Agriculture, Australia
	31	Diana B. Ingraham, AT International, Washington, D.C.
Apr.	15	Yves Crozat, ANGERS, France
	21	Ezio Andreta, Commission of the European Communities, Brussels, Belgium.
	27	Ronal Gallimore, Commission of the European Communities, Brussels, Belgium.
May	8-11	Dr. Paul Singleton, NifTAL Project.
Jun.	5	Dr. J.A. Garner, AIT, Thailand.
Jun.	23	Mr. John Schiller, ACNARP, Thailand.
Aug.	13	Dr. Kauser Malik, Nuclear Institute for Agric. & Biology, Pakistan.
Sept.	3	Dr. Toshifumi Murakami, TARC, Japan.
		Dr. Takuma Gamo, National Institute of Agrobiological Resource, Japan.
		Dr. Mitsuaki Watanabe, TARC representative in Thailand.
	11	Dr. Masanori Mitsuchi, National Institute of Agro-Environmental Sciences, Japan.
		Dr. Katsushige Shiraishi, Japan International Cooperation Agency (JICA), Khonkaen, Thailand.
		Dr. K. Hayano, Kyushu Agricultural Experiment Station, Japan.

Visitors to the NifTAL Project:

Jan. 7	Francisco Medina, DESC Sociedad de Founento Industrial, Mexico
Jan. 9	Nancy Glover, NFTA, Waimanalo, Hawaii
	Siti Rustini, Department of Agriculture, Indonesia
	Siti Nuraini, Department of Agriculture, Indonesia
Jan. 27	Donald T. Tokuoka, Aiea, Hawaii
Feb. 13	President's Club (UH Foundation):
	Lilly Au
	Lorna Burger & Laura Hind, Department of Education (retired)
	Don & Jean Carlsmith, Carlsmith, Wichman, Case, Mukai & Ichiki
	Sungdai Choi, Edsung Foods
	Kay Chung
	Jack Corteway, Bank of Honolulu
	Gordon Damon, Gordon Damon Realtors
	Dr. Thomas & Henrietta Fujiwara
	Judge George & Jane Fukuoka (retired)
	Dr. Louis Herman
	Chinn & Betty Ho, Capital Investment
	Soo-Yong Hong, Consulate General of Korea
	Connie Hustace
	Pat Hustace
	Dr. Andrew & Jennie In, University of Hawaii (retired)
	Hazel Kauahikaua, Department of Education (retired)
	Nolan & Jane Kramer, Mid Pacific Airlines
	Jack & Edith Leong, Mutual Distributors
	Peggy Melim
	Ko Miyataki
	Dr. Roger & Agnes Ogata
	Inez Pai
	Gertrude Pak-Chong, Department of Education (retired)
	John H.R. Plews
	Adriyana Rowan & Laurel Finley, University of Hawaii (retired)
	Richard & Althea Sato, Richard M. Sato & Assoc.
	Edward Scripps, Scripps Howard
	Clarence & Dusty Short, C.R. Short Realty
	Albert & Caroline Simone, President, University of Hawaii
	Barbara Smith, University of Hawaii (retired)
	Ben & Shirley Taguchi, M & E Pacific, Inc.
	Steve & Susan Taussig, Chemical Consultants Int'l.
	Harold & Janice Walker, Ernst & Whinney
	Vincent & Maureen Yano
Feb. 18	F.H. Bormann, Yale University
	Bernard Bormann, USDA Forest Service Research, Juneau, Alaska
	John J. Slavics, AT International, Washington, D.C.
Feb. 19	Andrea Blumberg, AID, S&T (AGR), Washington, D.C.
Mar. 12	Joe & Mary Jeanne Bouton, Agronomy Dept., University of Georgia
Mar. 13	Owen Campbell, Regina Sask., Canada
Mar. 17	Henry Carey, Santa Fe, New Mexico
Mar. 24	Robert M. Zablotowicz, Allelix Inc.
	Pat Lasby, Ontario, Canada
Mar. 25	Wendy Tamashiro, Makawao, Hawaii

Apr.	1	R. Velaguleri, Batelle Columbus Labs, Ohio.
	16	Grade 1 students and instructors from Doris Todd Memorial Christian School.
	28	Diah Setyorini, Center for Soil Research, Bogor, Indonesia.
		Arsil Salem, Center for Soil Research, Bogor, Indonesia.
		Romano L. Adupa, Crop Science Dept., Marere University, Kampala, Uganda.
May	1	Keith Jones, Dept. of Biological Sciences, University of Lancaster, United Kingdom.
	14	John & Mary Prescott, Tustin Thrift & Loan, Santa Ana, California.
	18	Dr. Nick Wheeler, Weyerhauser Research Station, Centralia, Washington.
		Bill Monahan, Maui Economic Development Board, Kahului, Hawaii.
	26	Colleen Welty, Makawao, Hawaii.
		Mildred Hazama, Kahului, Hawaii
		Dr. Ed Caswell, Dept. of Pathology, University of Hawaii, Honolulu, Hawaii.
		Donna Meyer, Dept. of Pathology, University of Hawaii, Honolulu, Hawaii.
	28	Gary Stewart, NW Ag News Network, Seattle, Washington.
Jun.	4	C.N. Lee, Dept. of Animal Science, University of Hawaii, Honolulu, Hawaii.
		Ivan Bingenheimer, La Grande, Oregon.
		Glade Janes, La Grande, Oregon.
		Harold Schild, Tillamook, Oregon.
	16	Dr. John Wooley, Director, NSF Biological Resource Centers Program, Washington, D.C.
	26	Elaine Ng, San Ramon, California.
		Marie Kunimura, Kaneohe, Hawaii.
		Ann Sakamoto, Kailua, Hawaii.
		Maureen Yano, Honolulu, Hawaii.
	29	John Bishop, Marysville, Ohio.
Jul.	9	Mark Andrews, State Representative, Kula, Maui, Hawaii.
		Herbert Honda, State Representative, Wailuku, Maui, Hawaii.
	17	Jess Daniels, Centralia, Washington.
	22	Mazen Khassawneh, Jordan Valley Agr. Services Project, Jordan.
	30	R.K. Bhatnagar, Dept. of Science and Technology, New Delhi, India.
Aug.	11	Walter Harada, College of Tropical Agriculture and Human Resources, Univ. of Hawaii, Honolulu, Hawaii.
		Richard Kotake, Honolulu, Hawaii.
	11	Charles Serikawa, University of Hawaii, Honolulu, Hawaii.
	18	Sandy Muntague, Australia.
	20	Agnes Padre, Soil Micro., IRRI, Los Banos, Philippines.
		Willis and Nancy Zimwelt, Sacremento, California.
Sept.	4	J.K. Ladha, IRRI, Philippines.
Oct.	1	T.S. Gill, S&T/AGR, USAID, Washington, D.C.
		G. Uehara, Dept. of Agronomy & Soil, Univ. of Hawaii, Honolulu, Hawaii.
		Peter Goldsworthy, ISNAR, The Hague, Netherlands.
	16	Charlotte Nakamura, UH Cooperative Extension Service, Maui County, Hawaii.
	21	Charles T. Sorenson, Honolulu, Hawaii.
	22	Bob Wheeler, Honolulu, Hawaii.

Mark Cassey, San Francisco, California
A. Ah Sau, University of Hawaii, Honolulu, Hawaii.
Michael Yoneda, University of Hawaii, Honolulu, Hawaii.
David Melrose and the students of Seabury Hall (34), Maui, Hawaii.
Bruce Antelinan, Information Express, Palo Alto, California.
A.N. Sen, IARI, New Delhi, India.
Pua'ala Fisher, University of Hawaii, Honolulu, Hawaii.
Bill and Phyllis Pugh, Wright & Company, Washington, D.C.
Blake Vance, Honolulu, Hawaii.

APPENDIX VI

FAO/NifTAL INOCULANT PRODUCTION TRAINING COURSE

COURSE CONTENTS

Overview - Legume Inoculant Production Course
Isolation of Rhizobia for Nodules
Rhizobium - Microbiology, Classification and Use
History and Development of The Legume Inoculant Industry
Selection and processing of Carrier Material for Use in Inoculants
Methods of growing Rhizobia in Mass
Enumeration of Rhizobia
Inoculant Production
Growth and Survival of Rhizobia in Peat
Quality Control of Legume Inoculants
Alternate Methods of Inoculant Production
Methods of Using Legume Inoculants
Location and Management of a Legume Inoculant Production Plant
Compatibility of Rhizobia with Chemical pesticides and Fertilizers
Effect of Multi-strain Inoculation and Inoculation Rates on Rhizobium Strain
Selection of Rhizobia for use in Agriculture (N-fixation), Competitive and Environmental Conditions
Legume Nodulation as Influenced by Host, Environment, and Effectiveness of Association
Influence of High Temperatures on the Growth and Survival of Rhizobium spp. in peat Inoculants During Preparation, Storage, and Distribution
Factors Affecting the Response to Inoculation and Strain Competition
Application of Serological Methods to Inocualant Production
Production of Antisera
Measurement of N-fixation
Rhizobium Culture Preservation
Rhizobium Culture Collections

APPENDIX VII

International Travel

Who: B. Ben Bohlool
Where: Washington, D.C.
When: June 1-7, 1987

Who: Nantakorn Boonkerd
Where: Dhaka, Bangladesh
When: Feb. 6-20, 1987

Who: Nantakorn Boonkerd
Where: Indonesia, Canada, NCSU, Brazil
When: August 1-31, 1987

Who: Robert J. Davis
Where: Washington, D.C., Nepal, Bangladesh, Thailand, Burma, Indonesia, Sri Lanka, India, Pakistan, Egypt, Jordan, Washington, D.C.
When: Nov. 25 - Mar. 2, 1987

Who: Robert J. Davis
Where: Honolulu (UH Manoa), Thailand
When: March 4-15, 1987

Who: Heinz Hoben
Where: BNFRC Bangkok, Thailand
When: Feb. 20 - Mar. 31, 1987

Who: Joann P. Roskoski.
Where: Samarinda, Cisarua and Jakarta, Indonesia.
When: July 6 - August 1, 1987

Who: Joann P. Roskoski
Where: Brazil, Ecuador, Colombia
When: August 20 - September 4, 1987

Who: Paul Singleton
Where: Thailand and the Philippines
When: May 8-May 20, 1987

Who: Paul Singleton
Where: Kenya, Uganda, Zambia, India, Thailand, Philippines
When: Sept. 17 - October 27

Who: Padma Somasegaran
Where: BNFRC Bangkok, Thailand
When: Feb. 20 - Mar. 31, 1987

Who: B. Ben Bohlool, Paul Singleton, Padma Somasegaran, Heinz Hoben, Patty Nakao, Paul Woomer, Janice Thies, Thomas George and Maria Luz Caces.
Where: Quebec, Canada
When: August 8-15, 1987
Purpose: To present research posters and papers at 11th NARC.

APPENDIX VIII

Experimental Materials Kit Contents

400 growth pouches
50 flex straws
80 test tubes, 25mm diameter x 200mm
5 bottles N-free plant nutrient solution, stock solutions
1 bag Legume seeds each (*Glycine max; Trifolium subterraneum; Medicago sativa; Vicia sativa; Phaseolus vulgaris; Leucaena leucocephala; Macroptilium atropurpureum; Phaseolus lunatus*)
24 Rhizobia cultures for the host legumes provided
72 Silica gel vials
50 30cc plastic vials
1 200 g silica gel
1 Bag cotton for silica gel vials
1 Rack for silica gel vials
1 170-foot roll of 8 gauge wire
1 Bag of assorted fertilizers
12 50g packets of uninoculated sterile peat
1 Packet of gum arabic sticker
1 Manual (Methods in Legume Rhizobium Technology)
1 Envelope containing: (15 data sheets for soil MPN results; 15 data sheets for correlating direct counts with plant infection counts; 1 copy of MPN Protocol for NSF Cooperators; 1 copy Legume Inoculants and Their Use, Fao/NifTAL Handbook and 2 Soil and Plant Entry Permits Kit)
1 NifTAL Germplasm Catalog
1 NifTAL Antisera Catalog

APPENDIX IX

Personnel

A. Scientific Staff

1. Senior Staff

Ben Bohlool, Ph.D.	NifTAL Director
Nantakorn Boonkerd, Ph.D.	BNFRC Director (Thailand)
Robert Davis, Ph.D.	Head, International Outreach Section; Coordinator Asia Network Foreign Training Coordinator
Heinz Hoben, M.S.	
Francois Robert, Ph.D. (May-Sep)	Assistant Professor (Overload)
Joann Roskoski, Ph.D.	Associate Project Director; Latin America Coordinator
Paul Singleton, Ph.D.	Head of Research; Assistant Agronomist; Coordinator African Network
Padma Somasegaran, Ph.D.	Microbiologist/Training Course Director

2. Technical Staff

Wendy Asano, M.S.	Research Associate
Candy Barr, B.S.	Laboratory Assistant
Charles Bowers, M.S. (Jan-Jul)	Research Associate
Brian Duffy (June-Aug)	Temp Summer Research Assoc
Geoffrey Haines (from Feb)	Field Assistant
Janet Hudson, B.S.	Research Associate
Kevin Keane, B.S.	Research Associate
Michael Koterba, M.S.(Jul-)	Field Assistant
Aree Leelawantanapun,B.S.	Research Assistant (Thailand)
Kathy MacGlashan, B.S.	Research Associate
Jonathan Mann, B.S. (from May)	Research Associate
R. Bruce Martin, B.S.	Research Associate
Patty Nakao, B.S.	Research Associate
Dawn Olsen, B.S.	Research Associate
Anong Rattanoubol, B.S.	Research Assistant (Thailand)
Joseph Rourke, B.S.	Research Associate
Lalana Sansopha, B.S. (Jan-Jul)	Research Assistant (Thailand)
Pipat Sukha, B.S.	Research Assistant (Thailand)
Yaowanuch Singhathep, B.S.	Research Assistant (Thailand)
Leanne Tanouye, B.S. (Jan-Mar)	Research Associate
Jitraporn Tawatpan, M.S.	Research Assistant (Thailand)
Rungtip Tinnachartvanit, M.S.	Research Assistant (Thailand)
Thomas Walker (from Jul)	Field Assistant (Casual)
Paul Woomer, M.S.	Research Associate

B. Support Staff

1. Senior Staff on Payroll

Princess Ferguson, B.P.A.	Head, Communication Section
Benjaluck Lertbhuddhikul, B.B.A.	Information Officer (Thailand)
Anchalee Pawai, B.Econ.	Secretary (Thailand)
Karean Zukeran, A.A.	Project Manager

2. Junior Staff on Payroll

Mark Fleming (Jun-Jul)	Maintenance Person
Richard Gabrielson	Graphic Artist
Susan Hiraoka	Clerk Typist
Pam Hoopii	Utility Person
Chonticha Jansuda	Office Maid (Thailand)
Patricia Joaquin	Clerk Typist
James Landgraf (Jan-Jul)	Maintenance Person
Ade Nikaido	Account Clerk
Cynthia Perry	Office/Library Clerk
Aroon Rattanamanukul	Janitor (Thailand)
Ray Smart (From Aug)	Maintenance Person
Joe Soliven (Jan-Aug)	Groundskeeper
Michael Unverzagt (from Jul)	Maintenance Person

C. Graduate Students

1. M.Sc. Degree Candidates

Naseer Mirza
Tanya Thongnunui (Thailand)

2. Ph.D. Degree Candidates

Taufiqul Aziz (Jan-Jun)
Thomas George
Janice Pinchin
Maria Luz Caces (from Feb)