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ANNUAL REPORT
1982

NFTAL

On the cover . . .

NIFTAL

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

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

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

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

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

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

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

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

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

NITROGEN FIXATION BY TROPICAL AGRICULTURAL LEGUMES

ANNUAL REPORT 1982

USAID CONTRACT NUMBERS ta-C-1207
and DAN-0613-C-00-2064-00

July 1, 1981 - December 31, 1982

Dr. Jake Halliday, Principal Investigator

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

UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT

STATISTICAL SUMMARY

Title:	Maximizing Symbiotic Fixation of Nitrogen by Grain and Forage Legumes in the Tropics (NifTAL Project)
Contract:	B) DAN-0613-C-00-2064-00 Formerly: A) AID/ta-C-1207
Principal Investigator:	Jake Halliday
Contractor:	University of Hawaii Honolulu, Hawaii 96822
Contract Period:	A) June 1, 1975 - June 15, 1982 B) June 16, 1982 - December 31, 1982
Reporting Period:	July 1, 1981 - December 31, 1982
Total AID Funding to Date:	A) $3,853,672 B) $ 675,000
Total Expenditures and Obligations through Previous Contract Year:	A) $2,873,210.15 B) not applicable
Total Expenditures and Obligations for Current Year:	A) $ 980,461.85 B) $ 416,573.59
Estimated Expenditures for Next Contract Year:	A) not applicable B) $1,258,426.41

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SUMMARY OF ACCOMPLISHMENTS

The NifTAL *Rhizobium* Germplasm Resource has grown from 1500 to over 2,000. More strains for various nitrogen fixing trees species and under-exploited grain legumes have been added. This expanded collection contains isolates from economically important grain legumes, pulses, forages, and tree legumes. Requests came from 25 countries and over 240 cultures were distributed.

Cross inoculation experiments are being carried out with the genus Leucaena and research indicates the need for specific rhizobia for nodulation.

Two additional legumes have been added in the INLIT for a total of 16, with inoculants available for all. A total of 197 kg. of inoculants were produced in 1982.

Research was conducted to demonstrate the special attributes of peat using NifTAL's dilution techniques. The tested peat appears to have an excellent nutrient status such that no extra nutrients need to be added during inoculant preparation. Work is in progress to evaluate the shelf life of these inoculants and to further define the special attributes of peat by chemical and biochemical analysis.

The NifTAL Antiserum Bank has been expanded. Forty-four antisera are now available for the identification of all INLIT strains by agglutination and immunodiffusion.

Twenty-five of these antisera have been conjugated with fluorescein isothyocyanate (FITC) and are available to INLIT collaborators who want to use the fluorescent antibody technique (FA) for strain identification. During 1982, 85 antisera were distributed.

A workshop on nitrogen recycling in cropping systems involving legumes is being planned for 1983. NifTAL will

gain expertise in cropping systems and soil microbiology through the meeting of experts in these fields.

An intercropping experiment by a NifTAL-supported Ph.D. student showed satisfactory yields of forage legumes. Leucaena (K-8) yielded in 363 days a dry matter yield of 19.1 MT monocrop and 18.3 intercrop, and accumulated 675 and 648 kg. of N, respectively. Greenleaf Desmodium accumulated 21.8 and 19.8 DM and 588 and 509 kg. of N.

During 1982, the International Network of Legume Inoculation Trials (INLIT) continued. An administrative reorganization of the Network resulted in the formation of a Middle East Network along with the African, Asian, and Americas Networks. Over 760 experiments were sent to Asian cooperators alone. Data analysis has been completed for a number of cooperators. "B" and "C" experiments have received UH approval and a manual has been produced for these experiments.

NifTAL's six-week training course in legume/Rhizobium technology was held in Bangkok 1 Nov - 10 Dec, 1982, for 17 participants from SE Asia. An evaluation of the training was conducted with results to be finalized in 1983. Formative evaluations on the expanded training manual were undertaken in the form of a field test and by expert review.

A book based on papers from the Cali Workshop and a variety of scientific articles were published.

Three issues of the BNF Bulletin were produced and mailed out to 1500 persons and institutions. A 4-page, continuing, bibliographic update for LDC researchers has been added. The document collection was expanded to 6000 publications, and over 360 document requests were filled. Five slide-tape programs were developed.

General Background

NifTAL's goal is to increase agricultural productivity throughout the tropics while minimizing dependence on costly, petroleum-dependent fertilizers by promoting effective utilization of the legume/*Rhizobium* symbiosis. The value of legumes in low-input cropping systems encompasses their high protein content when used as food, feed, or forage and their commercial versatility in areas such as timber, wood products, fiber, gum, soil improvement, and erosion control. They obtain their N through association with the beneficial bacteria, *Rhizobium*, and can also improve soil fertility for companion or following crops. NifTAL's strategy is to:

1. Identify effective strains of *Rhizobium*;

2. Demonstrate the benefits of *Rhizobium* inoculants by field trials throughout the tropics;

3. Provide technical assistance to institutions trying to maximize biological nitrogen fixation by legumes through training of key personnel in *Rhizobium* technology, through provision of germplasm inoculum or information resources or through expert consultancies in inoculant production.

Contracted Objectives

To achieve increased food production by more effectively exploiting the legume-*Rhizobium* symbiosis, NifTAL's objectives include the development of:

1. a. A collection of *Rhizobium* strains, including strains which have been tested and proven on targeted legumes, for distribution to researchers worldwide;

   b. Improved inoculum delivery techniques that ensure effective, dependable field inoculation; and

   c. Improved techniques and cultural systems to exploit more fully the nitrogen contribution of legumes in tropical cropping systems.

2. A formal network of cooperators in the tropics for field testing of *Rhizobium* inoculants.

3. Training programs on applied research and technology of *Rhizobium* bacteriology and agronomy for technicians and scientists in tropical countries.

Rhizobium Germplasm Resource

The number of *Rhizobium* strains has grown from 1500 to over 2,000. More strains for various nitrogen fixing trees species and underexploited grain legumes (bambara groundnut, horse-gram, tepary bean, mexican yam-bean, etc.) have been added. Over 400 special strains of rhizobia from soils with pH 4.5, 7.0, and 9.0 were isolated from root nodules of *Leucaena leucocephala*, *Arachis hypogaea* (peanut), *Desmodium* sp., *Stylosanthes guianensis*, and *Psophocarpus tetragonolobus* (winged bean). Rhizobia from nodules of winged bean grown in Guam soils have been added to the germplasm. Over 75 isolates from legumes found in Tunisia are being authenticated.

All 45 rhizobial strains used for experimentation in the International Network of Legume Inoculation Trials (INLIT) have been lyophilized in ampoules. These strains are for 15 different legume species. Thirty to fifty ampoules of lyophilized cultures have been prepared for each of the 45 strains of rhizobia. Work is in progress to complete freeze drying all cultures in the *Rhizobium* germplasm resource.

Another 35 legume species of potential importance have been selected so that *Rhizobium* strain selection can be initiated to provide a set of three effective and antigenically distinct strains for each of the legume species.

Information on all the rhizobia maintained in the germplasm collection has been computerized. A *Rhizobium* catalogue is being prepared for publication in 1983.

Culture requests came in from 25 countries. (See Table 1.) There were a total of 60 requests and 241 cultures were sent out.

Germplasm Research

Six species are known within the genus Leucaena: L. leucocephala, L. diversifolia, L. shanonni, L. lanceolata, L. retusa, and L. pulverulenta. The NifTAL Rhizobium germplasm stocks cultures for L. leucocephala, but no well tested cultures for the other species. The cross inoculation experiments using highly effective rhizobia (TAL 1145, TAL 582, and TAL 600) on L. retusa, L. pulverulenta and to some extent L. shanonni indicated the need for specific rhizobia for nodulation (Tables 2 and 3). Effective strains are being sought for these strain specific species.

ANTISERA

The NifTAL Antiserum Bank has been expanded. Forty-four antisera are now available for the identification of all INLIT strains by agglutination and immunodiffusion (See Table 4).

Twenty-five of these antisera have been conjugated with fluorescein isothyocyanate (FITC) and are available to INLIT collaborators who want to use the fluorescent antibody technique (FA) for strain identification.

A moderate amount of FITC conjugated Goat-Anti-Rabbit Globulin (GARG) has been stocked. This makes it possible to identify all INLIT strains by the indirect FA technique at NifTAL.

Work is underway to increase the total volume of all the INLIT antisera.

A facility will be set up for the production of GARG. Plans call for the production of sufficient FITC conjugated GARG to supply INLIT collaborators with this material together with requested antisera. In addition, FITC conjugated GARG will be made available

to workers who have antisera of their own but lack the facilties and equipment to produce GARG.

During the past year, there has been an increase in the number of requests for antisera. Antisera distribution is shown in Table 5.

INOCULANT DEVELOPMENT

Previous research (NifTAL Project Annual Report 1981) demonstrated that diluted (1000x) liquid cultures of Rhizobium can multiply and survive in two different peats (Nitragin peat, U.S.A., and Badenoch peat, Australia), indicating that peat had special attributes. This initial work involved only two strains of rhizobia (R. japonicum and R. phaseoli). It was necessary to demonstrate the special attributes of peat using the dilution technique over a wider range of rhizobial strains and carriers.

Results (Table 6) indicated that six of the seven strains of rhizobia tested could multiply to maximum numbers and survive satisfactorily up to 24 weeks. This indicates that peat was adequate in nutrients, supporting the use of diluted liquid cultures of Rhiozbium for inoculant production. Except for the cowpea Rhizobium (TAL 169), all other rhizobia reached ceiling populations in excess of 1 X 10!D9!U rhizobia per g. of moist peat after two weeks of incubation at 28 degrees C.

Three strains of rhizobia (R. phaseoli, R. japonicum, and Rhizobium sp. from Leucaeana) were used to further confirm attributes of peat by using peat from Mexico, Argentina, Peru, and Spain. The peat (Nitragin) from the U.S.A. was used as a standard. The results (Table 7) showed excellent multiplication and survival in both autoclaved and irradiated peats from the

different countries. Work is in progress to evaluate the shelf-life of these inoculants.

The data accumulated to date confirms the special attributes of peat as having an excellent nutrient status such that no extra nutrients need be added during inoculant preparation. Further work will be undertaken to define the special attributes of peat by chemical and biochemical analysis.

Legume Inoculant Production

Two more legumes added to the list of legumes being tested in the INLIT raised the total to 16. Inoculants are presently available for these legumes.

INLIT inoculants are being produced by the pilot plant. A total of 197 kg. of inoculants were produced in 1982.

Presterilized (gamma-irradiated) peat is being used for inoculant production and the quality has been excellent with viable counts ranging from $1X10^9$ - $1X10^{10}$ rhizobia per g of moist peat.

CROPPING SYSTEMS

Intercropping Workshop

Intensive planning was underway as of December for a workshop to be held in conjunction with Rodale Farms. The theme of the workshop is the recycling of nitogen in cropping systems involving legumes. NifTAL will thereby gain expertise in cropping systems and soil microbiology (including N recycling). We will design a model specifically for the purpose of estimating gains and losses to the croppng system. The dates are July 13-15, 1983, attendance will be by invitation only.

Future Cooperative N-Experiment

A ^{15}N experiment is also planned for March 1983 in cooperation with the joint FAO/IAEA Program. Two (November and January) corn crops must be planted first because the soil is rich in nitrogen. Four pasture legumes will be planted to ascertain how much N is being fixed in competition with a grass. A grass control will be added for comparison.

Intercropping Experiment

An intercropping experiment has been conducted for three seasons involving 2 forage legumes (Leucaena leucocephala cv Hawaiian giant, and Desmodium intortum cv Greenleaf) and 3 grain legumes--an indeterminate and a determinate mungbean (both from AVRDC in Taiwan) and soybean cv Davis.

During the cool season with reduced solar radiation, most of the plants had slower growth, with the exception of Greenleaf Desmodium.

At the final harvest (Aug 82, Table 8) the corn yields were all above those plots with no N (all in percentages): Leucaena 180, Desmodium 133, Mungbean determinate 269, indeterminate 216, and soybean 174, supplying 8, 4, 16, 11, and 8 kg. of N to the corn, respectively.

Season 1 (Oct 81) and season 3 (Aug 82) were very similar except for intercropped soybean (Table 9) which yielded much lower in season 1 than season 3 (1.0 metric ton (MT) vs. 2.8 MT). This was probably due to bird damage in the intercropped soybeans.

The yields of the forage legumes were satisfactory. In 363 days, Leucaena (K-8) yielded 19.1 MT monocrop and 18.3 MT intercrop and accumulated 675 and 648 kg. of N, respectively. Greenleaf Desmodium accumulated 21.8 monocrop and 19.8 intercrop MT and accumulated 588 and 509 kg. of N, respectively.

INLIT

The implementation of field trials continued during 1982. Formal agreements to continue INLIT trials still remain in many countries. India, the largest consumer of inoculants, receives its material in three large, seasonal shipments. Each shipment is disbursed by an Indian network coordinator. Where possible, all INLIT trials are mailed directly to cooperators in other countries. INLIT trials are planted at NifTAL to provide information on various germplasm which show local or regional promise.

Network Continuation

The Outreach Section continues to provide information and support to INLIT. NifTAL's staff helped design the analysis of INLIT experimental information which is returned after analysis to the coordinators and their network cooperators.

Although more experiments were sent out than data received, the pace of data receipts has been increasing. One continuing problem has been getting cooperators to send data back to NifTAL for analysis. Researchers have shown a tendancy to analyze their own experiments or keep the data in raw form for their own information.

To facilitate the information flow a form letter for the analysis of the "A" experiment was designed. It is hoped that this, coupled with two successive bulletins to the network cooperators explaining the statistical design of the experiment, will increase data input to the INLIT program. The quick turnaround time from receipt of raw data at NifTAL to receipt of complete analysis by individual researchers will add to increased participation.

Three "A" experiments were planted at NifTAL for comparative studies. One used Indian germplasm of the Cajanus species, while the other two used local seed varieties. An additional purpose of these trials was training NifTAL interns in certain agronomic techniques.

"B" and "C" Experiments

A "B" (strain testing) experiment has been produced with University of Hawaii departmental approval. The basic design has 12 treatments for comparing NifTAL and local inoculants under local conditions. The experiment was designed in consultation with the NifTAL statistician. Three trial runs on various "B" experimental designs using *Cicer arietinum*, *Phaseolus vulgaris*, and *Cajanus cajan* have been conducted at the NifTAL site. This work was done in cooperation with two NifTAL trainees to refine and test suitability of the experiment. The results suggested that at the NifTAL site the network inoculants are as good as any available. Since its approval, several "B" experiments have been sent out to various cooperators. To date, no results have been received. Appendix A presents the table of contents for the "B" and "C" experiments.

Asian Network

The Asian Network continued to grow during 1981-82. Over 760 experiments were sent to cooperators. These included some experiments to new cooperators and some repeat experiments. (See Tables 10 to 13.)

NifTAL staff continued to provide technical advice and services by visiting most of the countries where INLIT trials exist or where the possibility of establishing a BNF program remains high priority. Listed below are countries visited and a brief description of each visit's purpose.

Burma - To analyze legume inoculant production at Yezin and make suggestions toward improvement of production.

Sri Lanka - To discuss and make recommendations on training, research, and in-country legume inoculation work.

Malaysia (MARDI) - To provide technical advice on establishing field trials.

Taiwan (AVRDC) - To promote INLIT trials and provide technical advice on setting up experiments that will be acceptable to them.

Philippines (IRRI & PCARR) - To discuss the progress on INLIT trials.

Guam and the Northern Marianas - To survey for winged bean Rhizobium.

African Network

Eleven completed "A" experiments have been returned from the African region and at least five more are known to be planted out of the 36 experiments distributed. During this period the African region was divided into two areas. Northern Africa has been joined with the Middle East region to form the Middle East Network.

Middle East Network

Two experiments were conducted in Saudi Arabia, one alfalfa and one soybean. They were completed in June and August, respectively. A former NifTAL trainee was involved in these experiments.

A soybean trial was completed in Sudan (Wad Medani) in September but the cooperator has not yet submitted results.

Trials of lentil and chickpea have been installed in Egypt with the help of a former NifTAL graduate assistant. The lentil trials are in the Cairo area and upper Egypt, and the chickpeas are in middle Egypt, south of Cairo. The results will be ready in 1983.

NifTAL staff followed up on linkages during a trip to Cairo in 1982.

Americas Network

INLIT trials were promoted on a limited basis in Latin America and the Caribbean during this reporting period due to the early 1982 transfer of responsibility for this area from a departing staff member to the present coordinator. Support, however, was given to existing cooperators. Several INLIT "A" experiments were returned to NifTAL for data analysis.

COMMUNICATION AND TRAINING

NifTAL/Thailand Department of Agriculture Training Course

NifTAL's six-week training course in Rhizobium/legume technology was held in Bangkok, 1 November - 10 December 1982. The course was sponsored by USAID, the Thailand Department of Agriculture, the University of New England (Armidale, Australia), and the American Committee for Scientific Cooperation with Vietnam. A list of participants may be found in Appendix B.

Dr. Padmanabhan Somasegaran was the course director and Mr. Heinz Hoben was training director. Mrs. Yenchai Vasuvat, Director of the Thai Dept. of Agriculture's Rhizobium and Inoculant Production Project, and Dr. Nantakorn Boonkerd, Soil Microbiologist, made the training arrangements in Thailand and provided logistical support during the course. Visitors and guest lecturers included Dr. Joe Burton, Inoculation Production Specialist at NifTAL, Dr. John Thompson, Chief Pulse Microbiologist at ICRISAT, Dr. Doug Gross, Professor, Crop Science, North Carolina State University, Dr. Lloyd Frederick, Project Manager, USAID, and Dr. Benjavan Rerkasem, Lecturer, Faculty of Agriculture, Chiang Mai University.

Intern Training

Training was provided at NifTAL for 10 interns from 10 countries, totalling 37 months of training (see Appendix B).

NifTAL/ICRISAT Course

Preliminary discussions were started with ICRISAT regarding a 1983 training course in inoculant technology, to be held in Hyderabad, India. This course would be a combination of the six week basic legume/Rhizobium technology course and an expansion of current training exercises dealing with inoculant production and quality control.

Information on Training

Requests from over 100 individuals and institutions in 30 countries for information on training were answered by NifTAL.

Print Materials

Training Manual - The expanded version of the NifTAL training manual was field tested during the Thailand training course. It was also sent to approximately fifteen expert, external technical reviewers. The current version contains 21 detailed exercises, material lists, photographs and illustrations, and an expanded appendix.

Cali Workshop Proceedings - Based on papers presented at the 1981 International Workshop on Biological Nitrogen Fixation Technology for Tropical Agriculture, CIAT, Cali, Colombia, a 700-page book with over 70 articles was published by CIAT with assistance from NifTAL. The book's table of contents is presented in Appendix C.

NifTAL mailed over 150 copies to the Workshop's participants, and is currently selling the book to requesters at cost ($20 plus postage).

BNF Bulletin - Three issues of the BNF Bulletin were produced and disseminated to 4,700 individuals and institutions during 1981-82. The Bulletin has been standardized to eight pages with specific sections on research, meetings, personnel, books, and communication. The current BNF Bulletin mailing list includes approximately 1,500 persons.

A 3-4 page insert in the BNF Bulletin has been included. The insert is a continuing bibliographic update on the legume/Rhizobium symbiosis. All documents are numbered so readers in LDCs can request copies from NifTAL if the article is not available from the author (whose address is also included in the insert). A page from the bibliography is shown in Appendix D.

INLIT Manuals - INLIT B and C manuals were developed and are awaiting technical revision.

A small 4-page coloring brochure on legumes and Rhizobium was developed for children, aged 6-9. Another 4-page brochure was developed for lay persons visiting NifTAL.

Document Collection and Provision

During 1981-82, NifTAL requested over 400 scientific documents. The current collections contain over 6000 articles and books on the legume/Rhizobium symbiosis.

NifTAL answered 361 requests for documents in 1982 from over 60 countries.

Audio-Visual Programs

Several slide tape programs have been developed. These include:

a. What is NifTAL?--A layman's guide to the NifTAL Project.

b. The Old Maui High School--a pictorial history of the NifTAL facility on Maui.

c. Pasture Legumes: How to Inoculate--a description of legume/Rhizobium technology as applied to pastures and a step by step process on inoculation.

d. Pastures--a pictorial tour of pastures, emphasizing common legumes.

e. Nitrogen Fixing Trees--a quick look at over 30 N-fixing trees on Maui, plus an overview of the NifTAL project and its N-fixing tree component.

Sets of slides on NifTAL were also sent to the University of Hawaii and to USAID.

Visitors and Outreach

Over 150 visitors came to NifTAL during the second half of 1981 and 1982. A visitor list is included in Appendix E.

For the University of Hawaii's 75th Anniversary, NifTAL coordinated the public relations for UH agriculture related institutions on Maui. This included publicity and the hosting of an open house for the Maui community. A NifTAL display was also presented on the UH/Manoa campus during the anniversary celebration.

NifTAL had a manned display at the Maui County Fair to publicize the project to the community.

NifTAL also provided a display for UH's Mealani Field Day, held at Waimea, Hawaii, on pasture legumes and inoculation.

Research Abstracts and information on research work contained in Appendix F.

Travel Information on staff travel (dates of travel, destinations, and purpose) are included in Appendix G.

Personnel Staff personnel and an organizational organogram are included in Appendix H.

Scope of Work The scope of work and the 1983 work plan may be found in Appendix J. Planned outputs are also listed.

Statement of Expenditures and Obligations
and Contractor Resources

June 16, 1982 - December 31, 1982

Expenditures and Obligations by Input

CATEGORY	AMOUNT
Salaries & Wages	$184,883.68
Fringe Benefits	30,037.41
Consultants	1,050.00
Networks	8,423,41
Travel & Subsistence	22,494.06
Regional BNF Center	---
Participant Costs	28,201.87
Freight	6,886.08
Publications	2,411.58
Equipment/Vehicles/Supplies (Utilities/Facilities Included)	83,261.96
Subcontracts	---
Indirect	48,923.54
TOTAL	$416,573.59

Expenditures and Obligations by Output

Objective	Amount
1. Germplasm	62,486.03
2. Antisera	20,828.68
3. INLIT*	145,800.76
4. Inoculants	41,657.36
5. Crop Systems	20,828.68
6. Soil Fertility	---
7. Economics of BNF	---
8. Regional Centres	20,828.68
9. Training	104,143.40
TOTAL	$416,573.59

*International Network of Legume Inoculation Trials

Budget Forecast

January 1, 1983 - December 31, 1983

Estimated Expenditures and Obligations by Input:

CATEGORY	AMOUNT
Salaries & Wages	$472,716.32
Fringe Benefits	101,662.59
Consultants	5,950.00
Networks	122,576.59
Travel & Subsistence	34,505.94
Regional BNF Center	60,000.00
Participant Costs	53,798.13
Freight	1,113.92
Publications	22,588.42
Equipment/Vehicles/Supplies (Utilities/Facilities Included)	181,571.04
Subcontracts	53,000.00
Indirect	148,943,46
TOTAL	$1,258,426.41

According to Outputs

Objective	Amount
1. Germplasm	$ 143,194.65
2. Antisera	37,521.49
3. INLIT*	378,418.94
4. Inoculants	105,242.54
5. Crop Systems	68,561.05
6. Soil Fertility	26,500.00
7. Economics of BNF	26,500.00
8. Regional Centres	159,381.39
9. Training	313,106.35
TOTAL	$1,258,426.41

*International Network of Legume Inoculation Trials

TABLES
and
APPENDICES

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Table 1. 1982 *Rhizobium* Culture Requests

COUNTRY	REQUESTS	NUMBER OF CULTURES SENT
Argentina	2	27
Austria	1	11
Brazil	2	4
Colombia	1	2
Costa Rica	2	4
England	1	4
Ethiopia	1	17
Ecuador	1	3
Guatemala	1	9
India	1	1
Kenya	2	7
Mauritius	1	3
Mexico	3	28
Nigeria	1	6
Puerto Rico	1	3
Scotland	1	3
Senegal	1	10
South Africa	1	1
Sri Lanka	2	8
Thailand	3	37
U.S.A.	23	110
Uruguay	1	1
Venezuela	3	27
West Indies	3	13
Zambia	1	2
25	60	241

Table 2. Time to initial nodulation in the different species of *Leucaena* as affected by host x *Rhizobium* interaction.

HOST SPECIES	DAYS TO INITIAL NODULATION		
	STRAIN TAL 1145	STRAIN TAL 582	STRAIN TAl 600
L. leucocephala (K8)	10.5	10.5	10
L. diversifolia	10.5	11.25	10.75
L. shanonni	11.5	0	14.25
L. lanceolata	11.0	10	10
L. retusa	0	0	0
L. pulverulenta	16.5**	14*	15.5**

* Indicates nodulation in only one replication.
** Indicates nodulation in only two replications.

Table 3. Number of nodules on the different species of *Leucaena* grown in growth pouches at 24 days as affected by host X *Rhizobium* strain interaction.

HOST SPECIES	NUMBER OF NODULES PER PLANT		
	STRAIN TAL 1145	STRAIN TAL 582	STRAIN TAL 600
L. leucocephala (K8)	16.75	13.75	15.75
L. diversifolia	17.50	8.0	22.0
L. shanonni	10.0	0	5.75
L. lanceolata	21.0	19.75	21.0
L. retusa	0	0	0
L. pulverulenta	0.5**	1.25*	4.5**

* Indicates nodulation in only one replication.
** Indicates nodulation in only two replications.

Table 4. Distribution of Antisera in 1982

COUNTRY	REQUESTS	NUMBER SENT*
Australia	1	3
Egypt	2	19
Ethiopia	1	6
India	2	3
Indonesia	2	6
Malaysia	1	3
Nigeria	1	1
Philippines	1	3
Taiwan	1	9
Thailand	1	6
Trinidad	1	1
Turkey	1	3
USA	5	23
TOTAL	20	85

* Of each requested antiserum, 3-4 ml are generally sent.

Table 5. NifTAL Serum Bank

LEGUME SPECIES	TAL #	AGG*	ID**	FA***
Arachis hypogaea	1000	X	X	X
	169	X	X	
	1371	X	X	X
Centrocema pubescens	655	X	X	X
	651	X	X	
	1146	X	X	
Cicer arietinum	620	X	X	X
	480	X	X	X
	1148	X	X	X
Desmodium uncinatum	569	X	X	X
	1147	X	X	
	667	X	X	
Glycine max	102	X	X	X
	377	X	X	X
	379	X	X	X
Lens culinaris	634	X	X	X
	638	X	X	X
	640	X	X	X
Leucaeana leucocephala	82	X	X	
	1145	X	X	X
	582	X	X	X
Medicago sativa	380	X	X	X
	1372	X	X	
	1373	X	X	
Phaseolus vulgaris	182	X	X	X
	1383	X	X	
	1376	X	X	
Stylosanthes guianensis	309	X	X	X
	310	X	X	
	658	X	X	
Vigna radiata	441	X	X	
	420	X	X	
	169	X	X	X
Vigna Unguiculata	209	X	X	X
	173	X	X	
	658	X	X	X
Cajanus cajan	569	X	X	
	1127	X	X	X
	1132	X	X	X
Vicia Faba	1397	X	X	
	1399	X	X	
	1400	X	X	
Vigna mungo	309	X	X	
	570	X	X	
	999	X	X	X
Pisum sativum	1401	X	X	
	1402	X	X	
	1403	X	X	

* Agglutinations
** Immunodiffusions
*** Fluorescent Antibodies
NOTE: Purified gammaglobulins not listed since they are a step in other processes.

Table 6. Shelf life of inoculants prepared from autoclaved peat and diluted (with millipore sterilized water) liquid cultures of *Rhizoibum*.

RHIZOBIA*	LOG_{10} NUMBER OF RHIZOBIA PER G MOIST PEAT AFTER (WEEKS)				
	2	3	8	16	24
Rhizobium sp. (TAL 82)	9.45	8.92	8.71	8.23	7.89
Rhizobium sp. (TAL 169)	6.26	8.29	9.14	8.88	8.77
R. japonicum (TAL 379)	9.77	9.72	9.51	9.31	9.16
R. meliloti (TAL 380)	9.62	9.77	9.11	8.70	8.65
Rhizobium sp. (TAL 620)	9.41	9.61	9.30	8.94	8.72
Rhizobium sp. (TAL 651)	9.41	9.45	9.22	9.02	8.89
R. phaseoli (TAL 1376)	9.35	9.08	8.10	7.67	7.67

* Rhizobial isolates were from the following legumes: TAL 82 from *Leucaena leucocephala*; TAL 169 from *V. unguiculata*; TAL 379 from *Glycine max*; TAL 380 for *Medicago sativa*; TAL 620 from *Cicer arietinum*; TAL 651 from *Calopogonium mucunoides*; TAL 1376 from *Phaseolus vulgaris*.

Table 7. Growth and survival of rhizobia in peat inoculants prepared with liquid cultures diluted in millipore sterilized water. Inoculants were incubated at 28 degrees C.

	LOG_{10} NUMBER OF RHIZOBIA PER G MOIST PEAT			
	R. phaseoli (TAL 182)			
	AUTOCLAVED*		GAMMA-IRRADIATED*	
PEAT SOURCE	2 w**	4 w	2 w	4 w
Mexico	8.61	8.58	9.30	9.39
Argentina	9.06	9.07	9.51	9.70
Spain	8.99	9.19	9.17	9.50
Peru	nd.+	nd.	9.64	9.65
USA (Nitragin)	8.90	8.97	8.91	8.59

	LOG_{10} NUMBER OF RHIZOBIA PER G MOIST PEAT			
	R. japonicum (TAL 102)			
	AUTOCLAVED*		GAMMA-IRRADIATED*	
Peat Source	2 w**	4 w	2 w	4 w
Mexico	9.00	9.20	9.47	9.55
Argentina	9.43	9.58	9.67	9.86
Spain	9.31	9.19	9.39	9.48
Peru	9.23	9.20	nd.	nd.
USA (Nitragin)	9.37	9.52	9.39	9.48

	LOG_{10} NUMBER OF RHIZOBIA PER G MOIST PEAT			
	Rhizobium (TAL 82)***			
	AUTOCLAVED*		GAMMA-IRRADIATED*	
Peat Source	2 w**	4 w	2 w	4 w
Mexico	9.02	9.02	9.56	9.47
Argentina	9.25	9.26	9.88	9.70
Spain	9.38	9.18	9.58	9.32
Peru	nd.	nd.	nd.	nd.
USA (Nitragin)	9.28	9.05	8.96	9.05

* Indicates method of presterilization of the peat prior to injection of the diluted cultures. 50g quantities of the peat were autoclaved (with open ends folded down and held with a paper clip) in polypropylene bags for 60 minutes; bags were heat sealed upon cooling. Similar qunatities of peat were gamma irradiated (5 Mrads) in heat sealed polyethylene bags.

** Indicates time in weeks.

*** *Rhizobium* sp. isolated from *Leucaena leucocephala*.

+ Denotes not done.

Table 8. Corn Yields and Percentage of the Control Plots.

TREATMENTS	CORN YIELDS IN KG/HA AND PERCENTAGE OF CONTROL					
	OCT 81		MAR 82*		AUG 82	
	kg/ha	%	kg/ha	%	kg/ha	%
Control	388	100	363	100	373	100
Leucaena	496	127	234	64	673	180
Desmodium	263	68	267	74	496	133
Mung-detr.	614	158	558	154	998	267
Mung-In.	633	163	424	116	804	216
Soybean	632	163	433	119	652	174

* unusually low yields because of January storms and reduced solar radiation.

TABLE 9. Seed Yields of the Grain Legumes

LEGUMES	SEASON 1 October 81	SEASON 3 August 82
	- - - - - - kg/ha - - - - - -	
Mungbean, Detr.		
Mono	2,000	1,820
Inter	1,440	1,010
Mungbean, Indetr.		
Mono	1,680	1,680
Inter	960	1,320
Soybean		
Mono	2,770	3,000
Inter	1,010	2,800

Table 10. Asia Experiment "A" By Country

COUNTRY	SENT OUT	RETURNED
Bangladesh	32	2
Burma	4	0
Fiji	5	3
Guam	1	0
India	617	43
Indonesia	5	3
Malaysia	3	4
Nepal	3	3
Philippines	41	1
Pakistan	25	4
Samoa	0	0
Sri Lanka	2	1
Taiwan	23	0
Thailand	2	1
Vietnam	2	2
TOTAL	765	67

Table 11. Asia Experiment "A" By Crop

CROP	SENT OUT	RETURNED
Arachis Hypogaea	37	3
Cajanus Cajan	84	4
Calapogonium sp.	0	1
Centrosema pubescens	1	0
Cicer arietinum	103	15
Desmodium intortum	2	0
Glycine max	39	14
Lens culinaris	80	13
Leucaena leucocephala	12	1
Medicago sativa	1	0
Phaseolus vulgaris	3	0
Pisum sativum	76	0
Pueraria sp.	0	1
Stylosanthes guianensis	0	0
Vicia faba	2	0
Vigna Radiata (+ *V. mungo*)	316	12
Vigna unguiculata	9	3
TOTAL	765	67

Table 12. Asia Cultures By Country

COUNTRY	SENT OUT
Bangladesh	27
Burma	12
Fiji	0
Guam	0
India	53
Indonesia	0
Malaysia	3
Nepal	0
Philippines	12
Pakistan	33
Samoa	0
Sri Lanka	0
Taiwan	3
Thailand	3
Vietnam	6
TOTAL	152

Table 13. Asia Cultures By Crop

CROP	SENT OUT
Arachis Hypogaea	15
Cajanus Cajan	22
Centrosema pubescens	0
Cicer arietinum	20
Desmodium intortum	0
Glycine max	24
Lens culinaris	3
Leucaena leucocephala	12
Lupinus spp.	2
Medicago sativa	3
Phaseolus vulgaris	3
Pisum sativum	3
Sesbania spp.	13
Stylosanthes guianensis	0
Trifolium spp.	3
Vicia faba	3
Vigna Radiata (+ *V. mungo*)	21
Vigna unguiculata	5
TOTAL	152

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Appendix E. NifTAL Visitor List

LIST OF VISITORS

Carl & Grace Hittle	INTsoy-University of Illinois
Hassan Moawad	National Research Center, Egypt
Y.R. Dominegues	Senegal
Peter P. Rotes	UH Manoa
G. Oblisami	India
J.H. Gent	Iowa
F.B. Cady	Ithaca, N.Y.
Robert H. Randolph	E.W. Resource Systems Institute, HI
Ron Kondo	State Rep.
Alvin M. Asher	Washington, D.C.
Irvin Asher	Washington, D.C.
Lloyd R. Frederick	Washington, D.C.
James King	Indianapolis, Ind.
Joe C. Burton	Milwaukee, Wisconsin
Don Munns	U.C. Davis, CA
Tom Lumpkin	U.H. Manoa
Stan	U.H. Manoa
S. Talukdar	Africa
Dan Shigeta	Maui
Ned	CTAHR
Hal McArthur	CTAHR
Yukio Kitagawa	HITAHR
Pam Morrison	HITAHR
J.M. Imawato	UH Manoa
Ellen Kondo	Div. of Ocean Safety & Hlth. Honolulu
Daniel Rollins	Maui
Serge Verniau	Italy
Rhonda Lincoln	Maui
Utako Ga	Maui
Mable Y. Sakuma	Maui
Betty Yamashita	CES-Maui
Nancy Yamashita	CES-Maui
John Pawley	CES-Maui
Clark Hashimoto	CES-Maui
Chris Stearn	Puerto Rico
Michael Greene	Washington, D.C.
Eduardo Schroder	Puerto Rico
H. Douglas Gross	Raleigh, N.C.
Deane F. Weber	Beltsville, MD
Kobe Shoji	A & B Inc. Maui
John	HC&S Maui
Steve Kijima	HC&S Maui
Charles A. Simkins	Rangoon-Burma
Dick Phillips	Hilo, HI
Ken Cassman	
Nasir A. Saidy	Maui
Alvin Tagomuri	MCC, Maui
Marcus Franda	New Delhi, India
Carol Dunsell	Minnesota

LIST OF VISITORS

Brian Phelps	Minnesota
Uron Eugen	Libreville/Gabon
Kristina Lindstrom	University of Helsinki, Finland
Nancy Troland Cushman	Connecticut
John H. Cushman, Lt. Gen. US Army (Ret.)	Bronxville, N.Y.
Yun Soong Jin-Biology Group	McKinley High School, Honolulu
Eugenie Oen	Indonesia
Robert R. Trumble	National Science Foundation, Wash., D.C.
Elizabeth Anderson	Maui
Ed Bartholomew	MCC, Maui
Stephanie Aveira	Maui
Kevin LaPorta	International Tree Crops, Winters, CA
Masayi Kawabe	Haiku Pensioners Club, Haiku
Phillip Giovannini	Resource Systems Inst., Honolulu
Ken G. MacDicken	Dept. of Agronomy. Honolulu
Ukia Urata	Dept. of Agronomy & Soil Science, U. of H.
Judith M. Lyman	Rockefeller Foundation, N.Y.
Val G. Carlos	TRC-Bliss II Philippines
Maning Gualberto	ECO Tech Center, Philippines
Cholee Douning	Hilo
Richard F. Hyde	Holcomb Research Inst., Indianapolis, IN
U Khin Win	Agri. Corp. Rangoon/Burma
Dr. Kyi Win	Agri. Corp. Rangoon/Burma
U Tin Heaing	Rangoon/Burma
Li Labang Naw	Burma
Susan Shen	Office of Technology Assessment, Wash., D.C
Bruce Ross	Office of Technology Assessment, Wash., D.C
Craig Whitesell	U.S. Forest Service, Honolulu
M.S. Singh Deo	Agricultural Production Commissioner, India
M.D. Tedia	Director (Production & Research) M.P. State Oil Seed Federation, India
Maureen Kilkenny	University of Minnesota
David Lanter	LAWR U C Davis
Julie Hobenberg	LAWR U C Davis
Scott Cunningham	LAWR U C Davis
Doug Beck	U C D LAWR
Benjamin Oruko	U C Davis
Jeffery Bold	Davis, CA
Foster Cady	Ithaca, N.Y.
Vincent Sogle	California
Rob Woolfenden	Honolulu
Jim Maloney	MRI Midwest Research Inst. Kansas City, MO
Valerie Hammond	Honolulu
Joe & Aileen Ikeda	Utah
Harold & Janet Shiratori	Maui
J. Raymond Carpenter	Islamabad, Dept. of State, Wash., D.C.
Jose Germano Stammel	Porto Alegre-RS-Brazil
J.R. Jardim Fruic	Porto Alegre-RS-Brazil

LIST OF VISITORS

Carolyn Block & Kevin	Maui
Robert G. Megarrim	CSIPO Davies Lab Townsville, Australia
Jim Dillard	CSUF Fresno, CA
Bill Coops	RCUH Honolulu
Nelson Yuen	RCUH Honolulu
Jim Maloney	RCUH Honolulu
Tejpal S. Gill	A.I.D. Washington, D.C.
Raymond Jones	CSIRO Davies Lab, Australia
Liang-Ping Lin	Dept. Agr. Chemistry, Taipei
Kimie Tanaka	Maui
Robert Matsuoka	Maui
Fumi Takumi	Maui
Shigeko Kawamoto	Honolulu
Aiko Murata	Honolulu
Kimi Hazama	Maui
Sumi Okawa	Honolulu
Mabel Okazaki	Honolulu
Sui Lun Chee	Maui
Margaret Tamura	Honolulu
Hong Kwun (Wong) Pang	Honolulu
Robert Ooye	Los Angeles, CA
Hajime Nishimura	Maui
Noriyuki Ueoka	Maui
Irene Wienist Wery	Honolulu
Takashi Takimoto	Los Angeles, CA
Carla Valarik	Maui
Jill Engledow	The Maui News
Jock Anderson	Univ of New England, Australia
Jim Roumasset	Econ Univ Hawaii, East West Center
The Brewbakers	Kailua, HI
Lester Chon	Wailuku Sugar Co.
Harold E. Kauffman	INTSOY, IL
J.C. Nolan Jr.	UH, Honolulu
Cornelia Flores	Manhattan, KS
Warren R. Grant	Texas A&M (USDA) TX
Gary Hartshorn	Tropical Science Center, Costa Rica
Todd Aaron	Maui
Betty & Lowell Hill	University of Ill., Urbana, IL
Jerry Stockdale	Univ. Northern Iowa, Cedar Falls, IA
Jerry & Eileen Klonglan	Ames, Iowa
Rachel & Harold Breimyer	Columbia, MO
Bob Grossman	Honolulu
Narong Chomchalow	TISTR, Thailand
Srivan Chomchalow	TISTR, Thailand
Kovith Yautasash	TISTR, Thailand
Bill Eger	Maui
Adisak Sassapongse	AVRDC, Taiwan
Yuk, Chang-Soo	Director of Graduate School, College of Agri. Chungbuk National Univ., Korea
Cynthia L. Connolly	Int. Fertilizer Dev. Center, AL
Zane & Diana Hebel	Dept. of Agronomy, Univ of Missouri, MO
Richard Stowe	Maui

BIOLOGICAL NITROGEN FIXATION:

-- WHAT'S IN IT FOR HAWAII?

Jake Halliday

University of Hawaii NifTAL Project
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Nitrogen - the key to life

Nitrogen is an essential component of all life forms, being a cornerstone in the chemical structure of proteins. Nitrogen is abundant in the atmosphere and the air that we breath is 80% nitrogen. In this gaseous form, however, nitrogen occurs as dinitrogen molecules, each having two nitrogen atoms joined by a triple bond. The triple bond confers great strength to the linkage between the nitrogen atoms, making the nitrogen molecule one of the most unreactive compounds known. In this stable, gaseous form, nitrogen cannot be used directly.

Thus life on earth is completely dependent on continued transformation of atmospheric nitrogen into compounds that can be used readily by plants, and subsequently by animals and man. The process by which this happens is called nitrogen "fixation."

Paper presented at the Hawaii Fertilizers and Ornamentals Short Course, held at Kona, Hawaii during Jan 12-14 1983.

BIOLOGICAL NITROGEN FOR PASTURES

J. Halliday, J.W. King, and P. Nakao[1]

Many pasture soils are deficient in nitrogen in the form used by forage crops. However, some soil bacteria — which farmers may themselves introduce — have the ability to work with forage legumes to convert nitrogen from the air into a fertilizer-like form that the plant can use.

The biological process: The soil bacteria use a biological process to convert the nitrogen from the air into a form that is available to the plant. This biological process is a mutual association — called symbiotic — between the legume and the bacteria — called Rhizobium. There are many varieties or strains of Rhizobium which produce varying effects with different legumes. The Rhizobium bacteria have the ability to "infect" the roots of legume crops. After the rhizobia infect the roots, tumor-like nodules form to house the bacteria. The plant's photosynthesis process provides the Rhizobium with energy. In turn, through a complex biochemical process, the Rhizobium bacteria provide the plant with nitrogen.

The symbiotic process — The Rhizobium's ability to provide nitrogen to the legume is very important to farmers. The symbiotic process depends on two factors: the presence in the soil of suitable Rhizobium, and the specific needs of the legumes. Many farmers increase the available nitrogen in their pastures by inoculating their legume forage corps with prepared rhizobia. The rhizobia are made available in abundant quantity by applying inoculant — the prepared Rhizobium mixed in a carrier — to the seed prior to planting (seed applied method). The bacteria can also be applied by metering the inoculant into the furrow during planting (indirect method). The inoculant contains strains of Rhizobium which have been screened and selected for their superior ability to provide nitrogen to particular forage legumes.

A mistaken idea: Many farmers have a mistaken idea — that pasture legumes are promiscuous, and that they will nodulate with all Rhizobium. With this misinformation, farmers may make the decision that they do not need to inoculate their legumes. Farmers may also decide not to inoculate their legumes because they have heard that tropical soils contain enough Rhizobium to work effectively with their forage crops.

However: While it is true that many pasture legumes are promiscuous in their ability to associate with Rhizobium, this does not mean that the plant/Rhizobium association is an effective one. In fact, the

Paper presented at the 17th Mealani Beef Cattle Field Day, Mealani Experiment Station, Kamuela, HI, Oct 16, 1982.

PRESENT CONSTRAINTS TO, AND A FUTURE STRATEGY FOR, FULLER UTILIZATION OF LEGUME-FIXED NITROGEN FOR CROP PRODUCTION IN THE TROPICS

Jake Halliday

ABSTRACT

Legume crops are especially attractive when sustained productivity is sought from low-input farming systems in the tropics and subtropics because of their unusual ability to be self-sufficient for nitrogen supply. Life on earth is dependent on transformations of atmospheric nitrogen to a form in which it can be absorbed from soil by plants for protein synthesis. The process can be accomplished industrially but at a very high energy cost. Biological nitrogen fixation (BNF) by symbiotic associations of plants with microorganisms is economically more sound and environmentally more acceptable than nitrogen fertilizer use in agriculture.

Agrotechnology based on BNF by legumes has two facets, the use of legumes and inoculant technology. Currently legumes are used in many systems without any specific attempt to maximize their nitrogen fixation through inoculant technology. Yields can be increased and nitrogen fertilizer requirements reduced through implementation of appropriate inoculant technology. Maximum gains from BNF in agriculture will arise from innovative use of legumes in areas and in roles they have not occupied previously, provided that their nodulation and nitrogen fixation is assured. Most legumes in the tropics 'fix' about 100 kg/ha/year of nitrogen with levels around 350 kg/ha/year common for the forage tree leucaena and fixation as high as 800 kg/ha/year within the potential of some species. Fertilizer savings through the use of legumes represent not only significant savings in foreign exchange but also reduced dependence on the oil-rich nations whose influence over the cost and availability of nitrogen fertilizer is increasing.

The use of legumes involves the management of legume species in farming systems not only for direct benefits accruing from the multiple uses of legume products and the greater stability of mixed-cropping, as opposed to monocrop systems, but also for indirect benefits arising from their ability in some circumstances to make a net contribution of nitrogen to the soil and thereby to companion or following non-leguminous crops.

The underlying objective of inoculant technology is to introduce sufficiently high numbers of preselected strains of rhizobia into the vicinity of the emerging root that they have a competitive advantage over any indigenous soil strains of lesser N-fixing ability in the formation of root-nodules. Inoculation technology involves: selection of strains of rhizobia that are compatible and effective N-fixers with particular legumes; multiplying selected strains to high population densities in bulk cultures; incorporating the liquid rhizobial cultures into a carrier material (usually finely milled peat) for packaging and distribution; and finally, coating the seeds of legumes with the carrier or implanting the soil with the inoculant directly into the seed drill.

Legumes are already used widely and consistently, but as minor crops, in farming systems of the tropics. Inoculant technology is in use on a meaningful scale only in a few countries other than the U.S. and Australia.

Greatest future potential would appear to rest in the development of: legume-based pastures and viable multiple-cropping systems including legumes for under-utilized savannahs; agroforestry systems that combine fast-growing, nitrogen-fixing trees, leguminous and other crops to meet the food and fuel requirements of the rural poor; fast-growing leguminous trees for reclothing water catchment areas following forest clearance; legume-based cropping systems to give sustained productivity in cleared jungle soils which typically exhibit a rapid decline in fertility under conventional cropping; selection of deep-rooted, drought-tolerant leguminous trees that can serve as browse species in the world's dry lands.

The major constraints to full implementation of legume-based BNF technology in the tropics relate to the delivery and acceptability of the technology at the farm level, and are political, cultural, socioeconomic and, to a lesser extent, scientific in nature. The major scientific constraint is inadequate understanding of host legume x rhizobial strain x environment interactions resulting in inability to predict whether a given legume will respond to inoculation in a particular region or not. A constraint to deriving a better understanding of these interactions is the lack of trained personnel in the tropical regions to execute legume inoculation trials for determining the economic yield benefit attributable to legume inoculation. A lack of domestic inoculant production plants in the majority of countries also constrains research, development and production enterprises with legumes.

The scenario in which legume-based BNF technology would realize its potential would include: increasing economic and political pressure for greater energy efficiency in agriculture; increased recognition, by decision makers in funding agencies and in governments, of the potentials for exploiting BNF in the agricultural sector of the economies of developing countries; an increase in the cadre of trained professionals and technicians in countries of the tropics; improved integration of legume germplasm improvement programs and legume bacteriology programs, concerted application of international funding to catalyze establishment of a BNF Resource Center staffed, equipped and budgeted to provide technical assistance, offer germplasm and information services, provide professional and technical training and conduct such research as may be necessary for adaptation of BNF technology to individual needs of developing countries but which is beyond the current capability of local researchers; improved opportunity for interchange of findings from field research programs; implementation of a competently executed sequence of standardized experiments designed to quantify the economic yield benefit attributable to legume inoculation under field conditions and followed up by studies to quantify the nitrogen balance in multiple cropping systems that include legumes.

Legume inoculation does not substantially affect the need for farm labor. The inoculation is accomplished as an integral part of the sowing method whether by hand or mechanized planter. If fertilizers are normally applied, elimination of the need for nitrogen reduced the capital cost but no substantial labor saving is realized as other fertilizers still need to be applied. The use of legumes to benefit companion or following crops is consistent with the farming systems already prevalent in the tropics. To use legume-fixed nitrogen for, as an example, cereal production in the U.S. would necessitate adoption of mixed-cropping systems that are not easily mechanized. Thus an increased demand for labor would be an impact. The major positive impacts of BNF technology are indirect through elimination of the multitude of negative environmental and economic impacts associated with nitrogen fertilizer production, distribution and use in agriculture.

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PRACTICAL STEPS FOR SEED PREPARATION OF PASTURE LEGUMES

J. Halliday, P. Nakao, and J.W. King[1]

Introduction: The successful establishment of pasture legumes is affected by several factors in the seed preparation process. This presentation suggests practical steps to ensure (1) maximum germination of legume seeds, and (2) early nodulation of the plant roots by Rhizobium bacteria for enhanced biological nitrogen fixation. First, let us examine seed quality.

Seed quality: One of the parameters of seed quality is the germination rate. The germination rate is usually tested by the commercial seed producer and the results are reported on the label. Assuming the seeds have been kept dry and stored in a cool place, the seeds can retain the same level of viability for several months.

There may be a question about a particular batch of seeds because of prolonged or improper storage. A simple method to test the germination is to first place a small quantity of seeds (about 50) in warm water, soak overnight, rinse, and drain. The seeds can then be arranged between layers of clean, moist paper toweling and kept in a dark place. The toweling should be kept moist and the seeds checked each day. Depending on the species, the viable seeds should germinate after one to four days. The germination percentage can then be calculated and the rate of sowing adjusted accordingly.

Seed scarification to assist germination: The first step in the germination process is the swelling of the seed as the seed absorbs water. Some legume seeds, such as siratro, Centrosema, and some Desmodiums, have hard seed coats to protect them against long periods of adverse conditions. These seeds will not germinate readily unless they are first scarified, or "scratched," to allow for the absorption of water.

Usually commercial seed producers will mechanically scarify their seeds as necessary before selling them. To check whether a seed lot needs scarifying, soak a small sample overnight in warm water. If most of the seeds are not swollen after this soaking, the seed lot should be scarified.

Paper presented at the 17th Mealani Beef Cattle Field Day, Mealani Experiment Station, Kamuela, HI, Oct 16, 1982.

VIABILITY OF LYOPHILIZED CULTURES STORED FOR 37 YEARS

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Department of Agronomy and Soil Science, University of Hawaii, P.O. Box O, Paia, Hawaii, 96779

ABSTRACT

Lyophilization is considered one of the most satisfactory methods for the long-term preservation of microbial cultures. However, there is doubt about how long such cultures will remain viable. Appleman and Sears were the first to lyophilize cultures of Rhizobium. In 1940 they lyophilized several hundred cultures from the University of Illinois Soil Biology collection. After 3½ to 4 years, all of the Rhizobium cultures were still viable, and their ability to nodulate the host-plant and fix nitrogen were undiminished as compared with conventionally stored stock cultures. These cultures were later tested by Davis after 21 years of storage. At that time all 157 rhizobial cultures tested were viable and 138 of the 157 strains formed nodules. Those not forming nodules may not have been tested on the proper host plant since some were strains with a narrow host range. The lyophilized cultures have been tested for viability again after 37 years of storage. Out of 101 remaining strains of Rhizobium, 96 strains were viable. Nodulating ability was not checked on this occasion. The reduction in the number of strains surviving for 37 years compared to the data at 21 years indicate that viability of lyophilized culture does decrease, albeit slowly, during long-term stora

Proceedings of the 8th North American Rhizobium Conference, Aug 3-7, 1981, 1982.

Rhizobium inoculants for developing countries

Burton, J.C.
Hawaii University. College of Tropical Agriculture
Tropical Agriculture (Trinidad), v.58(4), 1981, 5p. : En
931061300
AID/ta-C-1207

The practice of culturing rhizobia to enhance the growth of legumes is increasing as the role of legumes as a major food protein supplier in developing countries gains importance. This report gives a broad account of the preparation, use, quality control, marketing, and distribution of *Rhizobium* inoculants for leguminous crops.

The rhizobia supplied by the inoculum infect root hairs of the host and cause nodules to form. Housed in these nodules, the rhizobia obtain food and energy from their host. The bacteria, in turn, gather or fix atmospheric nitrogen for plant growth. The general requirements of the *Rhizobium* strain are ability to form nitrogen-fixing nodules on the plant; competitiveness in nodule formation and survival in the presence of other infective rhizobia; prompt nodulation and good nitrogen fixation over a wide range of root temperatures; good growth ability in culture medium in the carrier and in soil; persistence or survival in the soil; and effectiveness on a wide range of host genotypes.

The rhizobia are usually mixed with a solid carrier which serves as their home until they are used by the farmer. Carriers provide a surface for growth of the bacteria and must permit gas exchange, particularly of oxygen. Food carrier material should be highly absorptive and easy to process, nontoxic to rhizobia, easy to sterilize, available in adequate amounts, inexpensive, and adhere well to seeds. Peat, charcoal, clay, bagasse, lignite, coal, compost, straw, and pulverized minerals such as vermiculite and apatite have been used as carriers with varying degrees of success.

The accepted method of using inoculants is to apply an aqueous slurry of the carrier base inoculant to seed just before planting. The amount of inoculum applied to seed varies from 2 to 25 g/kg of seed depending upon the brand and kind of legume seed. Soil inoculants (as opposed to seed inoculants) are usually more expensive but can be modified more easily for small farmers. An inoculant should be used within 4 to 6 months after it is made, and should be kept as cool as possible. As to proper distribution, inoculants must be handled as perishables since they contain live bacteria. Included is a 9-item bibliography (1932-79).

MODERN CONCEPTS IN LEGUME INOCULATION

J.C. Burton[1]

Summary

Inoculant technology has advanced considerably over the last century. Problems in host-range specificity have been addressed, and save for a limited number of tropical legumes, largely resolved. Rhizobia can be cultured to populations guaranteeing adequate numbers even in unsterile peat. While peat continues to be the favored inoculant carrier, various coal and compost formulations show promise for regions where the peat supply is inadequate. Processing or sterilization of the carrier is also being increasingly adopted. Thus, provided quality control is adequate, there is every reason to expect success in the routine inoculation of new lands or crops.

A number of new problems are emerging and will require intensive investigation, especially in the tropics. These include the failure of inoculant rhizobia to compete with native soil rhizobia in traditional areas of production for crops such as soybeans and beans, the need for an inoculant technology suited to the needs of the small farmer, and problems experienced as population pressure forces agriculture development into previously underexploited regions.

Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981, 1982.

Growth and N Fixation of Some Tropical Forage Legumes as Influenced by Solar Radiation Regimes[1]

F. I. Eriksen and A. S. Whitney[2]

ABSTRACT

Decreased solar radiation due to cloud cover or shading by plantation crops or associated grass can severely limit the production of tropical forage legumes. We therefore evaluated the response of six legumes (three replicated and three not replicated) to four radiation regimes (100, 70, 45, and 27% of unshaded solar radiation, hereafter termed "full sun") with polypropylene netting in the field.

The three replicated legumes had significant yield reductions at 27% full sun, with intermediate reductions at 70 and 45%. Dry matter (DM) yields at full sun [metric tons (mt) $ha^{-1}yr^{-1}$ and proportional yields at 27%] were: *Desmodium intortum* cv. Greenleaf (20.0, 46%); *Centrosema pubescens* 'centro' (13.7, 44%); and *Macroptilium atropurpureum* cv. Siratro (12.9, 20%). Greenleaf was relatively tolerant of moderate shading; proportional yields at 70 and 45% of full sun were 93 and 75%, respectively. Centro and Siratro yields declined linearly as shortwave radiation (hereafter termed "radiation") decreased, but Siratro yields declined significantly more than centro. Marked seasonal differences were noted in the response of the legumes to shade, and this was attributed to differences in the ability of the legumes to utilize solar radiation during periods of cool temperatures. The three non-replicated legumes were evaluated similarly, except that yields were adjusted for replication effects. Dry matter yields and proportional yields at 27% full sun were: *Leucaena leucocephala* cv. Hawaiian Giant (23.5, 40%); *Stylosanthes guianensis* cv. Schofield (17.0, 17%); and *Desmodium canum* 'kaimi clover' (12.2, 32%). Hawaiian Giant and Greenleaf yielded similarly at 45 and 27% full sun. Kaimi clover DM yield tended to be slightly higher (13.8) at 70% full sun, but thereafter yields declined linearly with reduced radiation. Schofield stylo was the most sensitive to shading.

Dry matter and N concentrations were not significantly elevated by reduced radiation or cool weather except that N increased during the cool season. Concentrations of N differed among species being highest for centro and Hawaiian Giant (3.4%) and lowest for kaimi clover (2.6%). Total N yields were associated with DM yields. Nitrogen yields (kg N $ha^{-1}yr^{-1}$) of replicated legumes at full sun and proportional yields at 27% full sun were: Greenleaf (540, 45%), centro (461, 44%), and Siratro (362, 27%); and for the non-replicated legumes, Hawaiian Giant (751, 38%), Schofield (496, 18%), and kaimi clover [340 (361 at 70% full sun), 38%]. Acetylene reduction rates by nodules in soil cores were highly correlated with radiation regimes ($r = 0.92$–0.995; except for centro, $r = 0.71$.) Correlation of acetylene reduction rates with DM yields ranged from 0.80 to 0.996.

The higher yielding legumes, Greenleaf, centro, and Hawaiian Giant (plus kaimi clover for ≈70% full sun only) appear well-suited for areas of low solar radiation because they have relatively constant concentrations of DM and N and fixed significant quantities of N even under dense shade.

Agronomy Journal, Vol. 74, July-August, 1982.

Effects of Light Intensity on Growth of Some Tropical Forage Species. I. Interaction of Light Intensity and Nitrogen Fertilization on Six Forage Grasses[1]

F. I. Eriksen and A. S. Whitney[2]

ABSTRACT

In order to determine the response of some tropical grass species to low light situations such as under plantation crops, seasonal cloud cover, etc., six tropical forage grasses were evaluated over a 20 month period on an Oxic Haplustoll in Hawaii (100 m above sea level) under four light regimes (100, 70, 45, and 27% daylight using polypropylene netting) in the field.

The forage grasses evaluated were: *Brachiaria brizantha*, *B. miliiformis*, *Digitaria decumbens*, *Panicum maximum*, *Pennisetum clandestinum*, and *P. purpureum*. Dry matter yields of N-fertilized (365 kg N ha^{-1} yr^{-1}) grasses were highest at 100 and 70% daylight (16 to 40 metric tons of dry matter (DM) ha^{-1} yr^{-1}), with *P. maximum* and *P. purpureum* having highest yields. Under 27% daylight, yields were 8 to 15 tons, with *P. maximum*, *B. brizantha*, and *B. miliiformis* having highest yields. When no N was added maximum yields in tons ha^{-1} yr^{-1} were *B. miliiformis*, 9.2 at 27% daylight; *D. decumbens*, *P. maximum*, and *B. brizantha*, 13.5 to 15.0 at 45% daylight; and *P. clandestinum*, 9.2 at 70% daylight. *P. purpureum* without N yielded 30 tons at full daylight, apparently because its very extensive root system invaded adjacent N-fertilized plots. Percentage of DM in the forage decreased with shading and N fertilization. Percent N increased with decreasing light intensity (from 1.0 to 1.6% in the minus-N and from 1.2 to 1.9% in the plus-N treatments). Slight acetylene reduction activity was found in soil cores beneath all species, except *B. brizantha*, indicating that the plants were almost entirely dependent on soil and fertilizer N for growth. Sward height increased significantly with decreasing light intensity and N fertilization. Concentrations of P, K, Ca, Mg, S, Cu, and Zn tended to be higher in shaded forage, higher in N-fertilized forage (except for P and Zn), and generally higher during the cool season.

Thus under N-deficient conditions, most yield and forage quality parameters were enhanced under moderate shade. Conversely, the tropical grasses studied generally responded to N fertilization only under conditions of moderate to high solar radiation.

Root weight data and observations on the rate of recovery after clipping indicate the shaded pastures would require careful management to avoid excessive depletion of root reserves, either by lenient grazing to maintain high leaf areas or by allowing an extended recovery period.

Agronomy Journal, Vol 73(3), May-June, 1981.

COMMUNICATION AND SCIENTIFIC INFORMATION IN AGRICULTURE: THE PROBLEM, THE ALTERNATIVES, AND THE FUTURE.

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When scientific information goes through the communication process, the result is often inaccuracies and a lack of comprehensiveness. This can be true when scientists communicate with scientists, and when scientists communicate with the general public. Mass communication is a significant problem in the area of agriculture. While a variety of alternatives to overcome these problems have been suggested, most of them encompass new and broader based media technologies. Generally overlooked are the basic communication components involved in planning based on systems concepts. Answers to six questions (1) What are the concerns? (2) Where can communication bring about change? (3) Who can bring about change? (4) What should be said? (5) How can we communicate? (6) What will be done to bring about change?, form the framework for systematic communication planning.

Two examples of the successful application of communication and scientific information in agriculture are the MIRCEN network and the NifTAL Project.

The future of communication and scientific information in agriculture is examined by studying future information availability and information demand, and four potential availability/demand interactions. Forces shaping these demands are noted -- population, income, environment, and limited natural resources. Given these forces, characteristics describing future oriented communication systems in agrotechnology are listed: innovative structures, access to information, interdependence, interdisciplinariness, and systems concepts.

Paper presented at a Unesco meeting, Edvard Kardelj University, Ljubljana, Yugoslavia, July, 1982.

THE TRAINING COMPONENT IN TROPICAL AGRICULTURE: A MANAGEMENT PERSPECTIVE

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SUMMARY

If agricultural development in the tropics is to keep pace with world demands, training will need to be an integral part of any organization's strategy. This paper describes the NifTAL Project, a tropical agricultural research project, and its training component, defines training, introduces the training process, and develops criteria for effective training. Types of commitments needed for training are presented, and, finally, training needs for tropical agriculture are noted.

Paper presented at the
"Tropical Agriculture Round-up:
New Partners for Pacific Food Security"
Asian-Pacific Agri-Energy Roundtable
Wailea, Hawaii, 9 December, 1982

QUANTIFICATION OF SYMBIOTIC NITROGEN FIXATION USING UREIDES: A REVIEW

D.L. McNeil[1]

Summary

This paper considers the genera of legumes known to produce ureides; the tissues on which ureide analysis can be made; and the advantages and disadvantages of ureide analysis as a means of estimating symbiotic nitrogen fixation.

INTRODUCTION

Ureide compounds in plants have been extensively studied, with numerous reviews on their biochemistry (Tracey, 1955; Bollard, 1959; Reinbothe & Mothes, 1962; Van der Drift, & Vogels, 1966) and production in legume nodules (Atkins, Rainbird & Pate, 1980; Woo, Atkins & Pate, 1980) already published.

In legumes, allantoin and allantoic acid are the principal ureides. These substances can be regarded as the ultimate product of nitrogen (N_2) fixation for a range of legume species (Matsumoto, Yatazawa & Yamamoto, 1977a) and are transported in the xylary sap to support plant growth (Israel & McClure, 1979). Both have a low C:N ratio, and so are conservative of carbon (C) relative to N when used in N storage or transport.

Measurement of the ureides found in nodulated legumes has attracted considerable interest in recent years, both as a means of estimating total N_2 fixation and for determination of the amount of N_2 fixed, relative to other sources of N. This approach assumes that the rate of xylary ureide output is a direct measure of N_2 fixation (McClure, Israel & Volk, 1980) whereas other sources of N for the plant are present as other compounds in the xylary sap (McClure & Israel, 1979). Measurement of ureide and non-ureide N would, thus, permit determination of the relative contribution of fixed and absorbed N to plant development.

Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981, 1982.

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Variations in Ability of *Rhizobium japonicum* Strains To Nodulate Soybeans and Maintain Fixation in the Presence of Nitrate

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Received 15 January 1982/Accepted 18 May 1982

This study investigated differences in sensitivity to nitrate of soybean (*Glycine max* cv. Davis) symbioses with 16 different *Rhizobium japonicum* strains. When nitrate (20 mM) was added to established symbioses, there were no significant differences in the degree of inhibition of acetylene reduction for any of the 16 strains. When nitrate was present during the establishment of nodules, high levels of nitrate (10 mM) were equally inhibitory on all symbioses, whereas specific strain effects appeared at low (0.5 mM) to medium (2.0 mM) levels of nitrate. At 1.5 mM nitrate in solution culture, the days to emergence of nodules varied from less than 10 (CB:1809, Nit61A118) to more than 16 (11 of 16 strains). In a clay-pot trial maintained at the low nitrate level (0.5 mM), symbioses with CB:1809 increased total nodule mass by 30% relative to nitrate-free controls. In the presence of 2.0 mM nitrate, CB:1809 maintained total nodule mass. For the remaining 6 strains tested, total nodule mass decreased to below the levels of the nitrate-free controls. In a separate clay pot trial, CB:1809 increased its competitive ability relative to USDA:110 when nitrate was added. If no nitrate was added, CB:1809 occupied 0.97 times as many nodules as USDA:110; when 10 mM nitrate was added, CB:1809 occupied 1.75 times as many nodules as USDA:110. Attempts to select nitrogen-adapted substrains of *R. japonicum* through sequential isolation and infection of plants grown on nitrate were not successful.

Soil acidity tolerance of symbiotic and nitrogen-fertilized soybeans

Munns, D.N.; Hohenberg, J.S.; Righetti, T. L.; Lauter, D. J.
Hawaii University. College of Tropical Agriculture
Agronomy Journal, v.73, 1981, 407-410p. : En
931061300
AID/ta-C-1207

Although acid soil greatly depresses growth in most legumes by impairing nodulation, this study presents evidence to indicate that for soybeans, aluminum (Al) toxicity inhibits growth more than does soil acidity. Two lime nitrogen (N) factorial trials with acid soils were conducted to observe lime effects on nodulation, early growth, and N concentrations in symbiotic soybean plants as compared with control plants. Each trial soil was high in exchangeable and soluble Al. In both trials, liming from pH 4.4 to 6.0 doubled growth, regardless of N source, cultivar, or *Rhizobium* strain or numbers. Inoculated plants were nodulated, green, and high in N even when their growth was severely acid-affected.

Symptomatic indications that soybean growth in the acid soils was limited by Al toxicity to the host plant were confirmed in solution culture experiments with pH, Al, and calcium (Ca) controlled at levels resembling those found in extracts of the soil solutions. Growth was unaffected by low Ca or low pH, but was depressed by Al.

These data suggest that efforts to improve acid tolerance in the soybean should center on plants, not rhizobia. Further, if the soybean can nodulate effectively in acid soil where its growth is severely limited by Al toxicity, then soybeans are an exception to the generality that legumes fail in acid soil because of poor nodulation. Consequently, it is justifiable to ignore rhizobia and supply N when screening for acid-soil tolerance in soybeans.

The authors conclude that although these findings are consistent with other field trials, there is evidence to imply that they can be expected only in the large group of acid soils where Al toxicity is the dominant limitation. A 14-item list of cited literature (1929-79) is appended.

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Effect of Salinity on *Rhizobium* Growth and Survival†

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Received 25 February 1982/Accepted 18 May 1982

This study examines the effect of salinity on the growth and survival of *Rhizobium* spp. in culture media and soil. Eleven isolates from saline and nonsaline environments were compared. The growth (mean doubling time) of all strains and species tested decreased when the electrical conductivity of the culture medium (yeast extract-mannitol) was raised from 1.2 mS cm^{-1} to 6.7 mS cm^{-1} (15% seawater equivalent) or to 13.1 mS cm^{-1} (28% seawater equivalent). Three of eleven strains failed to grow at 13.1 mS cm^{-1}. Although growth was affected by salinity, four strains selected from the growth rate study could survive in extremely high concentrations of salt. Two strains with growth rates sensitive to salt and two strains with growth rates relatively unaffected by salt were inoculated into solutions with electrical conductivities of up to 43.0 mS cm^{-1} (92% seawater equivalent). Not only did all four strains survive the initial osmotic shock (at 5 h after inoculation), but it was not until 27 days after inoculation that the sensitive strains exhibited a significant reduction in viable numbers. The salt-tolerant strains survived for more than 65 days with no reduction in viable counts. The interaction between soil moisture tension and soil salinity in relation to *Rhizobium* survival in gamma-irradiated soil was also examined. Six treatment combinations were used, ranging from −0.1 bars and 0.2 mS cm^{-1} to −15 bars and 12 mS cm^{-1}. Sensitive strains declined from 10^7 to 10^5 organisms per g of soil after 84 days of incubation at −15 bars and 12 mS cm^{-1}. Tolerant strains survived for the same period with no loss in viable numbers. The results of these experiments indicate that many strains of *Rhizobium* can grow and survive at salt concentrations which are inhibitory to most agricultural legumes. The emphasis of research concerning the effects of salinity on symbiotic nitrogen fixation should, therefore, be directed to aspects of the symbiosis other than the survival of the *Rhizobium* spp.

THE EFFECT OF SALINITY ON RHIZOBIUM SURVIVAL, NODULE FUNCTION AND NODULE FORMATION IN THE SOYBEAN-RHIZOBIUM JAPONICUM SYMBIOSIS

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN AGRONOMY AND SOIL SCIENCE

AUGUST 1982

By

Paul W. Singleton

ABSTRACT

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

The growth rate of Rhizobium in culture media is slowed by the addition of NaCl. Some strains were incapable of growth at the highest level of salt used (120 mM NaCl). However, all withstood substantial osmotic shock and most survived for extended periods in saline solutions equivalent to the concentration of sea water. The results show that the effects of increasing moisture tension and salinity on Rhizobium survival in soil are additive.

By independently subjecting nodules and shoots to salinity stress it was possible to show that the soybean-Rhizobium japonicum nodule system was not greatly affected by exposure to 120 mM NaCl. The main reduction in nitrogen fixation was the indirect effect of salinity on leaf expansion, shoot yield potential and the sink for nitrogen.

The early processes of nodule formation were extremely sensitive to NaCl in the rooting medium. When only 26.3 mM NaCl was added to the nutrient solution two hours prior to inoculation, nodule number and mass were reduced by 50% and 79.9 mM NaCl reduced nodule number, mass and nitrogen fixation to less than 10% of the controls. Rhizobium japonicum, reisolated from nodules from the high salt treatment did not form more nodules under saline conditions than isolates from controls.

By independently subjecting the various processes of the symbiosis to salinity stress it was determined that the early steps in nodule formation are the most sensitive to salinity. This sensitivity indicates that high quality irrigation water must be used during the establishment of symbiotic legumes.

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Comparison of the Pour, Spread, and Drop Plate Methods for Enumeration of *Rhizobium* spp. in Inoculants Made from Presterilized Peat†

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Received 9 March 1982/Accepted 15 July 1982

Inoculants prepared with presterilized peat were enumerated by the pour, spread, and drop plate techniques. Results indicated that the three plating methods were interchangeable. The drop plate method was preferred because of its economy in materials and labor.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1982, p. 330-333
0099-2240/82/080330-04$02.00/0
Vol. 44, No. 2

Dilution of Liquid *Rhizobium* Cultures To Increase Production Capacity of Inoculant Plants†

PADMANABHAN SOMASEGARAN* AND JAKE HALLIDAY

NifTAL Project, Department of Agronomy and Soil Science, University of Hawaii, Honolulu, Hawaii 96822

Received 16 February 1982/Accepted 27 April 1982

Experiments were undertaken to test whether peat-based legume seed inoculants, which are prepared with liquid cultures that have been deliberately diluted, can attain and sustain acceptable numbers of viable rhizobia. Liquid cultures of *Rhizobium japonicum* and *Rhizobium phaseoli* were diluted to give 10^8, 10^7, or 10^6 cells per ml, using either deionized water, quarter-strength yeast-mannitol broth, yeast-sucrose broth, or yeast-water. The variously diluted cultures were incorporated into gamma-irradiated peat, and the numbers of viable rhizobia were determined at intervals. In all of the inoculant formulations, the numbers of rhizobia reached similarly high ceiling values by 1 week after incorporation, irrespective not only of the number of cells added initially but also of the nature of the diluent. During week 1 of growth, similar multiplication patterns of the diluted liquid cultures were observed in two different peats. Numbers of rhizobia surviving in the various inoculant formulations were not markedly different after 6 months of storage at 28°C. The method of inoculant preparation did not affect the nitrogen fixation effectiveness of the *Rhizobium* strains.

NODULATION, NITROGEN FIXATION AND RHIZOBIUM STRAIN AFFINITIES IN THE GENUS LEUCAENA

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University of Hawaii NIFTAL Project
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ABSTRACT

Leucaena belongs in the subfamily Mimosoideae of the Leguminosae. All ten species have been reported to nodulate readily, but some doubt remains in the case of L. retusa which has failed to nodulate in Hawaii soils.

The infection process has not been studied in Leucaena. Nodule shape is variable, being Astragaloid, Desmodioid, or Muconoid in form. Attachment to the root is narrow and fragile.

Nitrogen accumulation rates of 600 kg/ha/year and as high as 1,000 kg/ha/year are on record, but annual nitrogen fixation has not been determined with precision. One approximation, based on the acetylene reduction technique, puts annual fixation at 110 ± 30 kg.

The specific designation for the leucaena rhizobia is Rhizobium loti (leucaena). These rhizobia are typically fast-growing, gummy and translucent on yeast-mannitol agar. They are neutral, or slightly acidic in their pH reaction on YMA. Leucaena rhizobia will nodulate other tropical legumes usually nodulated by fast-growing rhizobia, and will also nodulate some species normally nodulated by slow-growers. Slow-growing strains of Rhizobium from other tropical genera do not nodulate leucaena.

Leucaena usually nodulates spontaneously with indigenous soil rhizobia without specific inoculation, but there are sufficient documented cases of nodulation failure to prompt adoption of legume inoculation when leucaena is introduced to locations where it has not been grown before.

Although leucaena performs very poorly in acid soil sites, strains of Rhizobium for leucaena are available that exhibit tolerance to low pH. Acid tolerance in a strain is not dependent on an alkali-producing capability. One particular strain, TAL 1145, has proven to be competitive for nodule formation, persistent in the soil microflora and to be a fully effective nitrogen-fixing partner for leucaena in acid soils.

Paper presented at the Leucaena Workshop held in Singapore, Nov, 1982.

INTERNATIONALLY SPONSORED DEVELOPMENT OF BIOLOGICAL NITROGEN FIXATION TECHNOLOGY

Compiled by S. C. Harris[1]

from data of E.J. Da Silva,[2] L.R. Frederick,[3] F. Maignon,[4] G. Persley,[5] and F. Riveros[4]

Summary

Efforts of international funding agencies interested in promoting biological nitrogen fixation technology are increasing. These efforts center on training inoculant production personnel, on increasing the availability of germplasm and inoculants, and on field experiments to demonstrate the viability of inoculation, particularly with regard to the legume/ *Rhizobium* symbiosis.

THE ROLE OF LEGUMES IN MIXED PASTURES

A.S. Whitney[1]

Summary

Legumes can contribute greatly to pasture production by providing high protein forage, especially during the dry season when grass quality is poor. Little nitrogen (N) is transferred underground by actively growing legumes, but aboveground transfer of N, principally in legume leaf litter, green leaf which is trampled into the soil by grazing animals, urine from animals grazing legumes, and feces, is significant. Management alternatives to maximize the legume N contribution are discussed.

Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers Presented at a Workshop Held at CIAT, March 9-13, 1981. 1982.

Public Communication Campaigns. **Ronald E. Rice and William J. Paisley, editors. Beverly Hills: Sage Publications, 1981.**

James W. King, A review of Public Communications Campaigns, Vol. 65, No. 2, April-June, 1982.

APPENDIX G. International Travel

TRAVELLER	DATES OF TRAVEL	DESTINATION	PURPOSE
J. C. Burton	07/30-08/08/81	WINNIPEG, CANADA	To attend 8th North American Rhizobium conference, visit various AID projects enroute.
J. C. Burton	11/17-11/27/81	BANGKOK	To lecture at Thailand Institute in Science and Technological Advancement - MIRCEN (Microbiological Resources Center) course.
J. C. Burton	09/05-09/11/82	BANFF, CANADA	To attend "2nd International Symposium on N Fixation with Non-Legumes" conference.
J. C. Burton	11/09-12/10/82	BANGKOK/ANAHEIM, CA/RIVERSIDE, CA	To lecture at NifTAL's training course in Bangkok, attend ASA meeting in Anaheim & consult with & advise group at Riverside. working on USAID Tunisian Legumes.
E. Cuautle-Fabian	10/18-11/02/82	LIMA, PERU	To attend XI Latin American Rhizobium Conference to present papers and to represent NifTAL.
R. J. Davis	08/01-08/08/81	WINNIPEG, CANADA	To attend 8th North American Rhizobium Conference at the U/Manitoba.
R. J. Davis	08/16-08/24-81	FIJI/SIVA/APIA/ PAGO PAGO	To assess needs for training & other BNF activities in the S. Pacific, to meet w/ Robert Craig, USAID, & to discuss program w/personnel in the Dept. Agriculture & U.S. Pacific.

R. J. Davis	09/12-10/19/81	GUAM/PHILIPPINES/ BANGKOK/NEPAL/ DELHI/LAHORE/ ISLAMABAD/KARACHI/ FRANKFURT/ROME/ WA, D.C.	To establish linkages follow up on formerly made arrangements & plan experiments. To make contact with cooperating institutions in developing countries & to discuss the need for rhizobiology training in order to carry out NifTAL's contracted objectives of providing training to technicians in dev. countries.
R. J. Davis	10/31-12/19/81	TOKYO/HONG KONG/ MALAYSIA/INDONESIA/ THAILAND/ BANGLADESH/	To establish linkages, follow up on formerly made arrangeements & plan experiments relating to the INLIT, make contact w/cooperating institutions, & discuss training needs.
R. J. Davis	03/07-06/13/82	GUAM/TAIWAN/ PHILIPPINES/ MALAYSIA/SINGAPORE/ SRI LANKA/INDIA/ NEPAL/BURMA/ THAILAND/PAKISTAN/ NEW YORK/WA, D.C.	To establish linkages, follow up on formerly made arrangements & plan experiments. To also make contact w/cooperating institutions in order to carry out contracted objectives.
R. J. Davis	10/29-11/19/82	SRI LANKA	Served as rhizobiology consultant for Soybean Development Project at request of FAO, Rome.
S. Dowdle	08/01-08/10/82	WINNIPEG, CANADA	To attend 8th North American Rhizobium conference at U/Manitoba.

J. Halliday	09/27-10/03/81	TAIWAN	To participate as invited speaker in Workshop on BNF Exchange germplasm materials and seek participation in the INLIT.
J. Halliday	11/08-11/15/81	ARGENTINA	To attend National Workshop on BNF and present two invited papers. Visit centers for research on industrial fermentation & deliver a set of INLIT strains to government lab for national distribution.
J. Halliday	01/24-02/02/82	VENEZUELA	To teach Rhizobium strain selection in int'l course, to consult w/INLIT cooperators & to coordinate w/Dr. Munns, NifTAL sub-contractor, UC/Davis.
J. Halliday	09/15-10/05/82	WA/NY/MILAN/ROME	Visit NAS & USAID, Rockefeller & Donner Found., NY, run NFT Conference, Bellagio, Italy & cooperate w/ FAO BNF programs.
H. Hoben	10/10/82-01/05/83	SINGAPORE/MANILA	Conduct NifTAL train course in Thailand & visit various NifTAL Asian cooperators.
J. King	07/01-07/06/82	YUGOSLAVIA	Attend Problems & Challenges in Communicating Science Workshop and present
J. King	10/26-11/17/82	BANGKOK	To assist in conducting NifTAL training course.

D. McNeil	08/01-08/08/81	WINNIPEG, CANADA	To attend 8th North American Rhizobium Conference at U/Manitoba.
P. Singleton	10/23-11/03/82	LIMA, PERU	To attend XI Latin American Rhizobium Conference.
P. Somasegaran	08/01-08/08/81	WINNIPEG, CANADA	To attend 8th North American Rhizobium Conference at U/Manitoba & make two paper presentations on Inoculant Production and Serology.
P. Somasegaran	10/10/82-01/05/83	BANGKOK/MALAYSIA/ SINGAPORE/MANILA	To direct and teach in NifTAL's training course in Rhizobium Technology in Bangkok and to perform follow-up visits to former NifTAL trainees and cooperators.
A.S. Whitney	01/22-02/02/82	VIENNA, AUSTRIA	To attend IAEA/FAO research coordination meeting to define roles and research priorities for NifTAL as well as coordinate w/NifTAL as their future research objectives are closely related to NifTAL's.
A. S. Whitney	03/16-04/05/82	ANKARA/CAIRO	To coordinate INLIT trials w/coordinators in Middle East Region & attend the First OAU (Organization of African Unity) Conference
R. Woolfenden	08/01-08/16/82	WINNIPEG, CANADA	To attend 8th North American Rhizobium conference at U/Manitoba.

Appendix H. Personnel List

Position Title	Name
Principal Investigator/Director	Jake Halliday
Project Manager	Karean Zukeran
Associate Soil Scientist	Vacant
Network Microbiologist	Padma Somasegaran
Network Agronomist	Bob Davis
Assistant Agronomist	Paul Singleton
Inoculant Production Specialist (50% FTE)	Joe Burton
Soil Scientist, BNF Resource Center	Douglas Beck
Training/Communication Specialist	James King
Research Associate/ Training Coordinator	Heinz Hoben
Research Associate	Patty Nakao
Research Associate	Bruce Martin
Research Associate	Steve Borton/Judith Dozier
Research Associate	Janice Pinchin
Research Associate	Stephen Weekly
Laboratory Assistant (80% FTE)	Barbara Bose
Laboratory Assistant	Kathleen Kuailani
Field Assistant	Hank Anderson
Accounts Clerk	Adeline Nikaido
Courier/Clerk Typist	Paulette Cainglit
Data Processor Operator	Marylou Baker
Information Assistant	Princess Ferguson
Utility Person (80% FTE)	Pamela Hoopii
Groundskeeper/Security Officer (50% FTE)	Joseph Soliven
Graduate Assistant	Rob Woolfenden
Graduate Assistant	Evangelina Cuautle-Fabian
Graduate Assistant	Steve Dowdle
Graduate Assistant	Shiva Chaudhury

Appendix I. Scope of Work

Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL)

USAID Contract DAN-0613-00-2064-00
(Formerly AID/ta-C-1207)

June 16, 1982 - December 31, 1986

SCOPE OF WORK

A. PURPOSE

To reduce the dependence of LDC farmers on nitrogen fertilizer for the production of increased quantities of food by research and assistance to Rhizobium and legume programs throughout the tropics to more effectively exploit legume-Rhizobium symbiosis. Specific objectives to achieve the output are described in the Scope of Work below.

B. SPECIFIC OBJECTIVES

OBJECTIVE 1 (Germplasm)

Maintain a Rhizobium Germplasm Resource comprised of authenticated, characterized rhizobial strains which effectively nodulate agriculturally important legumes under the conditions prevailing in developing countries.

Activities

(1a) Augment the Rhizobium Germplasm Resource, to include specifically the following: strains for fast-growing leguminous trees of potential as browse species in seasonally dry range lands and/or for agroforestry systems; strains from highland locations in the tropics that are effective with temperature legumes of importance in cropping systems in developing countries; and additional strains isolated from tropical legumes growing under severe soil stresses (soil acidity, salinity, waterlogging, drought, and high temperatures).

(1b) Identify three strains of Rhizobium for each legume of actual or potential importance in developing countries which, if included in a mixed inoculant, would offer: diversity of origin with respect to host genotype and environment, and tolerances to tropical soil stresses (salinity, acidity, water stress, and high temperatures). When possible, the three strains will be serologically distinct.

(1c) Produce legume inoculants on a pilot scale for field testing at developing country locations and to support research and development programs with legumes in developing countries.

(1d) Distribute information on the characteristics and nitrogen fixation effectiveness of strains of Rhizobium to legume researchers throughout the tropics.

(1e) Provide cultures of Rhizobium requested by researchers and inoculant producers in the tropics.

OBJECTIVE 2 (Antisera)

Establish a serum bank housing antisera for identifying the strains of Rhizobium used in inoculants for the International Network of Legume Inoculation Trials (INLIT).

Activities

(2a) Develop antisera for identification of individual Rhizobium strains by agglutination, gel immunodiffusion, or fluorescent antibody procedures.

(2b) Distribute catalogue and information on antisera held in the serum bank.

(2c) Distribute lyophilized aliquots of antisera to INLIT cooperators and to researchers who request them for studying the competitive ability and persistence of INLIT rhizobial strains.

OBJECTIVE 3 (International Network of Legume Inoculation Trials/INLIT)

Determine the yield benefit resulting from the inoculation of local varieties of agriculturally important legumes under the soil and climatic conditions of the regions in the tropics where they are, or will be, grown.

Activities

(3a) Continue implementation of the three-stage sequence of trials (INLIT) developed and endorsed by international participants throughout the tropics.

(3b) Provide materials, technical advice and analytical services in support of the INLIT.

(3c) Perform statistical analyses and interpretation of the results from INLIT.

(3d) Distribute the findings of the INLIT in a range of information materials suited to widely differing clientele groups.

(3e) Develop approaches to solving region, or country-specific problems identified during the INLIT through workshops and technical assistance.

OBJECTIVE 4 (Inoculants)

Develop systems for improved inoculant delivery and for ensuring dependable, effective nodulation in the field.

Activities

(4a) Attempt to define more precisely the specific attributes of peat that have made it the most widely accepted carrier for rhizobia in legume inoculants.

(4b) Evaluate alternate carriers and packaging materials suitable for inoculant delivery in developing countries of the tropics.

(4c) Develop improved or novel techniques for assuring successful inoculation under biological stress (competition from indigenous soil rhizobia for nodulation of promiscuous legumes), chemical stress (soil acidity factors), and physical stress (high soil temperatures).

(4d) Test alternate inoculant production systems offering simplicity and economy of operation.

OBJECTIVE 5 (Crop Systems)

Develop improved techniques and cultural practices for increasing the nitrogen fixed and the contribution of nitrogen to companion or following non-leguminous crops in selected tropical cropping systems.

Activities

(5a) Develop methodologies for quantifying the nitrogen balance in tropical cropping systems that take account of: total nitrogen gain from biological nitrogen fixation; host-plant variation (specific and varietal) in nitrogen content of residues; the fraction of residue nitrogen added to soil organic matter; the proportion of residue nitrogen that is mineralized; the fraction of residue nitrogen taken up by companion/following crops; the percentage of residue nitrogen lost from the system; and the net change in soil nitrogen status.

(5b) Employ the standard methodologies developed in activity 5a for studying the nitrogen balance of a selected cropping system in the developing countries.

(5c) Assess the impact of management factors on the nitrogen balance in developing country cropping systems that include legumes.

(5d) Describe those elements of legume/non-legume competition in cropping systems that adversely affect nitrogen fixation by the legume.

OBJECTIVE 6 (Soil Fertility)

Study the effects of soil infertility on symbiotic performance of legumes in order to define tolerances, guide choice of legumes for agricultural systems, improve performance, and lower costs of soil management.

Activities

(6a) Define acid, salt, and phosphate tolerance of less-known legumes and their rhizobia; and screen for tolerance if justified.

(6b) Survey phosphate requirements of rhizobia; assess the likelihood that high phosphorus requirement causes field failure of otherwise effective strains and screen for tolerance of low phosphorus if justified.

(6c) Attempt to find out how salinity limits performance of legume symbioses that are important in less humid areas.

(6d) Attempt to find out how soil chemical stresses, such as hydrogen ion concentration and aluminum, interfere with division of rhizobia and with infection and nodulation.

(6e) Test the connection between tolerance exhibited by rhizobial strains and the conditions from which they are collected and isolated.

(6f) Test the validity of laboratory and greenhouse screening procedures as indicators of rhizobial performance under field conditions.

OBJECTIVE 7 (Economics of BNF)

Provide economic information on the relative costs of BNF technology and of fertilizer nitrogen technology through an economic analysis of the demand for legume inoculants.

Activities

(7a) Specify the farm model. The model is likely to be based on well-known budgeting and linear programming techniques.

(7b) Inventory available data.

(7c) Estimation of demand.

OBJECTIVE 8 (Regional Centres)

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

Activities

(8a) Identify a capable cooperative institution in at least one developing country prepared to host a Regional BNF Resource Center to provide for research, technical assistance and training support to the African, Asian, or Latin American regions.

(8b) Locate an outreach scientist specializing in BNF technology at the Regional BNF Center.

(8c) Install a pilot-scale inoculant production plant at the Center (if not already established).

(8d) Assist former NifTAL trainees now located in developing countries to design relevant production-oriented research programs and to obtain budgets to support their activities.

OBJECTIVE 9 (Training)

Train professional researchers, technicians, and extension workers for their respective roles in the development and implementation of legume-based BNF technologies.

Activities

(9a) Provide four (4) graduate research assistantships on a continuing basis to M.S. and Ph.D. candidates who undertake these projects that further NifTAL's research objectives and who, on completion of their training, will contribute to agricultural development in developing countries.

(9b) Conduct at least one further six-week training course in legume/*Rhizobium* technology that will be mounted in a developing country in cooperation with a host institution and, when possible, with the participation of additional sponsors.

(9c) Conduct training workshops for extension workers. One workshop to be mounted at the Regional BNF Resource Center and one workshop to be staged in a selected country of the region (other than the country hosting the BNF Resource Center). Existing capabilities of other institutions will be tapped through specific subcontracts when considered advantageous for implementing such workshops.

(9d) Conduct at least one training course on inoculant production at the Regional BNF Resource Center.

(9e) Offer intern training experiences at the NifTAL Project headquarters to key personnel from legume programs in developing countries.

(9f) Develop training materials in support of activities 9b, 9c, 9d and 9e and make these available for use by national institutions in developing countries.

Appendix J. Planned Output for Research Support Section

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UNIVERSITY OF HAWAII NIFTAL PROJECT
  Planned Outputs for Research Support Section  : 1983

                        : JAN   FEB   MAR   APR   MAY   JUN   JUL   AUG   SEP   OCT   NOV   DEC   JAN

OBJECTIVE 1 : GERMPLASM
                              <a1> priority/target strains lyophilized  <c> production ongoing
                              <a2> catalogue strains lyophilized        <d1> strain catalogue available
                              <b1> priority list defined                <e> services ongoing
                              <b2> target list defined
                              <b3> inoculnt strains recommended

  1-a) the collection   -----:-----:-----:-----:-----:-----:-<a1>:-----:-----:-----:-----:<a2>-:-----:-----:
  1-b) strain selection -----:-<b1>:<b2>-:-----:-----:<b3>-:-----:-----:-----:-----:-----:-----:-----:-----:
  1-c) inoculants       -----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:
  1-d) information      -----:-----:-----:-----:-----:<d1>-:-----:-----:-----:-----:-----:-----:-----:-----:
  1-e) services         -----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:
  -------------

OBJECTIVE 2 : ANTISERA
                              <a1> priority strains available           <b1> antisera catalogue
                              <a2> total available                      <c> services ongoing

  2-a) the bank         -----:-----:-<a1>:-----:-----:-----:-<a2>:-----:-----:-----:-----:-----:-----:-----:
  2-b) information      -----:-----:-----:-----:-----:-----:-<b1>:-----:-----:-----:-----:-----:-----:-----:
  2-c) services         -----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:
  -------------

OBJECTIVE 4 : INOCULANT DEVELOPMENT
                              <a1> publ.'Peat;properties,use'           <c1> compare inoculation methods
                              <a2> publ.'Peat;review'                   <c2> stress tolerant strains
                              <b1> review other carriers                <c3> non-sterile peat strains
                              <b2> composted material                   <c4> publications
                              <b3> review packaging                     <d1> integrated systems brochure
                              <b4> publ.'Carrier review'                <d2> peat-alternate processing
                              <b5> publ.'Packaging'                     <d3> multi-level system writeup

  4-a) peat             -----:-----:-----:-----:-----:-----:-<a1>:-----:-----:-----:-----:<a2>-:-----:-----:
  4-b) carrier/packging -----:-----:-----:<b1>-:-----:-----:-<b2>:-----:-<b3>:-----:-----:<b4/b5>----:-----:
  4-c) stress soils     -----:-----:-----:-----:-----:<c1>-:-----:-<c2>:-----:-<c3>:-----:<c4>-:-----:-----:
  4-d) productn systems -----:-----:-----:<d1>-:-----:-----:-<d2>:-----:-----:-----:-<d3>:-----:-----:-----:
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OBJECTIVE 5 : N-MANAGEMENT
                              <a1> workshop planning completed          <a3> publ.'Workshop proceedings'
                              <a2> N-management workshop                <b1> Hawaii field test preparation
                                                                        <b2> publ.'Hawaii field tests'
  5-a) Hawaii model     -----:-<a1>:-----:-----:-----:-----:-<a2>:-----:-----:-----:-----:-<a3>:-----:-----:
  5-b) LDC model        -----:-<b1>:-----:-----:-----:-----:-----:-----:-----:-----:-----:-<b2>:-----:-----:
  5-c) managmnt effects -----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:
  5-d) competitn effects-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:-----:
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