FINAL

Annual Report

1978-1979

ANNUAL REPORT

NifTAL Project
June 1, 1978 - June 30, 1979

Maximizing Symbiotic Fixation
of Nitrogen by Grain and Forage
Legumes of the Tropics

UH/AID Contract ta-C-1207

University of Hawaii
College of Tropical Agriculture
Department of Agronomy and Soil Science

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REPORT SUMMARY

Statistical Summary

Title:	Maximizing Symbiotic Fixation of Nitrogen by Grain and Forage Legumes in the Tropics (NifTAL Project)
Contract:	AID/ta-C-1207
Principal Investigator:	Jake Halliday
Contractor:	University of Hawaii Honolulu, Hawaii 96822
Contract Period:	June 1, 1975 - June 30, 1981
Reporting Period:	July 1, 1978 - June 30, 1979
Total AID Funding to Date:	$2,929,066
Total Expenditures and Obligations through Previous Contract Year:	$ 946,341
Total Expenditures and Obligations for Current Year:	$ 462,800
Estimated Expenditures for Next Contract Year:	$ 645,700

Project Personnel

Alphabetical List of Staff:

Staff	Position	Months Served
D. Billings	Research Assoc.	7/78 - 6/79
B. B. Bohlool	Assoc. Project Director Sr. Advisor, Microbiologist	7/78 - 3/79 3/79 - 6/79
B. Bose	Lab Assistant	7/78 - 6/79
J. Bose, II	Bibliographer	7/78 - 6/79
R. Bousquet	Secretary	4/79 - 6/79
K. G. Cassman	Grad. Research Asst.	7/78 - 5/79
J. De Coite	Janitor/Maintenance	1/79 - 6/79
S. H. Dworak	Editor	1/79 - 6/79
J. Halliday	Project Director Principal Investigator	 3/79 - 6/79
H. Hoben	Research Assoc. Training Coordinator	7/78 - 6/79 3/79 - 6/79
J. Kato	Research Assoc.	7/78 - 6/79
B. Kerrey	Project Manager	12/78 - 6/79
C. King	Student Asst.	1/79 - 6/79
R. B. Martin	Research Assoc.	3/78 - 6/79
R. S. Mawson	Research Assoc.	7/78 - 6/79
S. N. May	Grad. Research Asst.	7/78 - 6/79
P. Nakao	Research Assoc.	7/78 - 6/79
T. Ohara	Research Assoc.	7/78 - 9/78

Staff	Position	Months Served
V. G. Reyes	Soil Microbiologist	7/78 - 5/79
J. Rothenberg	Student Asst.	1/79 - 6/79
W. G. Sanford	Assoc. Project Director	7/78 - 2/79
P. W. Singleton	Grad. Research Asst.	7/78 - 6/79
P. Somasegaran	Soil Microbiologist	7/78 - 6/79
T. Stevens	Groundskeeper	4/79 - 6/79
K. R. Stockinger	Agronomist	7/78 - 6/79
A. S. Whitney	Project Director	
	Principal Investigator	7/78 - 3/79
	Sr. Advisor, Agronomist	3/79 - 6/79
P. Woomer	Grad. Research Asst.	7/78 - 6/79
K. Zukeran	Office Manager	7/78 - 6/79

Summary of Accomplishments

Since its inception in 1975, the NifTAL Project has assembled a *Rhizobium* germplasm collection of over 1,000 strains. Cultures have been screened for superior effectiveness in fixing nitrogen and for tolerance to stress conditions common in tropical soils. Inoculation techniques and cultural practices have been examined and selected to increase the level of symbiotically fixed nitrogen. Cropping systems have been studied for their ability to enhance the benefit of symbiotic nitrogen fixation in crop production. In the area of training, 24 technicians from 17 countries participated in four "*Rhizobium* Technology" courses at the NifTAL site.

In the past year, the reorganization of the germplasm collection was initiated to expand the number of strains held and to establish 1) a "classical" collection in lyophilized ampoules for long-term storage; and 2) a "working" collection on ceramic beads for medium-term storage and easy dissemination. Screening of superior strains was continued at NifTAL's Maui site, and work has continued on improved inoculation techniques and cultural practices for increased nitrogen fixation. Arrangements were made for summer training courses in Nairobi, Kenya and Porto Alegre, Brazil. A hands-on intern training program was established at the NifTAL site to accommodate trainees from areas not accommodated at the regional training courses. A manual for future training courses was written for use in this year's courses. An organizational workshop was held to plan a network of inoculant response experiments throughout the tropics.

ANNUAL RESEARCH REPORT

General Background

The NifTAL Project was funded by USAID as part of an effort to address two interdependent problems:

1) Insufficient food production in the tropics for a rapidly growing population; and

2) A diminishing, costly supply of the petroleum products used in the production of almost all synthetic nitrogen fertilizers for agricultural production.

NifTAL seeks to increase world food production while minimizing dependence on expensive, petroleum-dependent fertilizers. The approach is to maximize use of biological nitrogen fixation by selected strains of *Rhizobium* bacteria living in symbiotic association with tropical food and feed legumes. The NifTAL strategy has been to identify superior strains of *Rhizobium*, demonstrate the benefit of *Rhizobium* inoculants in tropical countries, provide technical training to key personnel in those countries, and provide technical expertise in inoculant production.

Contracted Objectives

To achieve increased food production by more effectively exploiting the legume-*Rhizobium* symbiosis, NifTAL has dedicated its time to the development of:

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

Improved inoculum delivery techniques to ensure effective, dependable field inoculation;

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

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

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

Priorities

NifTAL's objectives will remain the same over the balance of the contract period. However, a change in priorities will affect the amount of effort to be directed toward each objective. Greatest emphasis will now be placed on the establishment of a formal network

of cooperators in the tropics and on the development of the training program.

The collection of *Rhizobium* strains will be improved and expanded but will not receive the attention it enjoyed in the past. Work on delivery techniques will continue, and more time will be invested in the development of a pilot inoculant plant with a corresponding effort on guidelines for quality control of inoculum production.

Improved techniques and cultural systems to exploit the nitrogen contribution of the legume/bacteria symbiosis are very important, but budgetary constraints dictate that minimum manpower be employed in this area at NifTAL.

Annual Accomplishments

Following the plan of work outlined in the 1977-78 Annual Report, progress at the NifTAL Project proceeded in several directions.

Rhizobium *Collection and Screening*

The germplasm collection at NifTAL has been reorganized to separate the holdings into long-term storage (in lyophilized ampoules) and a working collection stored on ceramic beads.

Underexploited legumes: Certain legumes with untapped potential for food production occupy restricted niches in the tropics and have been virtually unknown as economic crops outside these areas. Their introduction into additional zones necessitates assuring the availability of effective and compatible *Rhizobium* strains for inoculation.

The underexploited legumes *Vigna aconitifolia, Vigna umbellata, Pachyrrhizus erosus, Dolichos lablab, Voandzeia subterranea* and *Phaseolus lunatus* were grown in Leonard jars after being inoculated with strains of *Rhizobium* isolated from a diverse array of legume genera. Wide variation in effectiveness of the symbiosis with different strains of *Rhizobium* was observed for each legume. *Phaseolus lunatus, Phaseolus acutifolius* and *Pachyrrhizus erosus* did not nodulate effectively with rhizobia from most of the legumes of the cowpea group. It is inferred that these three species would benefit from inoculation with specific rhizobia when introduced into new environments.

Pasture legumes: Improved production of legumes in pastures holds the promise of increased supply of livestock products for human consumption. The pasture legumes *Desmodium canum* and *Glycine wightii* were inoculated with a range of *Rhizobium* isolates and grown in Leonard jars. The *Rhizobium* strain isolated from *Desmodium canum* was the most effective in increasing the dry matter production of *Desmodium canum*. Broad spectrum strains TAL 169 and TAL 309 were fully effective in increasing top weights of *Glycine wightii*.

Screening for target grain legumes: Grain legumes that are staples in human diets receive high priority status in efforts to improve agricultural production. It is important to identify those strains of *Rhizobium* which are effective in fixing nitrogen for these legumes. *Phaseolus vulgaris, Vigna unguiculata, Cajanus cajan* and *Arachis hypogaea* were inoculated with various strains of *Rhizobium* and grown in Leonard jars to screen for superior strains.

Cultivar-by-strain interactions were examined in each of the four species. One strain, TAL 182, was effective on all the varieties, while another was effective only on two varieties. Six strains were effective on more than one cultivar.

TAL 1000, a strain isolated at the NifTAL Hamakuapoko site, proved most effective on both cultivars in the first experiment and most effective on three of twelve cultivars in the second experiment.

There were significant cultivar-by-strain interactions indicating that attention needs to be paid to selecting the proper strain for each variety.

Effectiveness of mutant strains: Antibiotic-resistant strains are useful as labels that identify strains of bacteria which have infected plant roots. Such mutant strains were developed at NifTAL by treating stock cultures with streptomycin, spectinomycin and a combination of streptomycin and spectinomycin. These strains were compared to their parent strains in greenhouse experiments to determine whether the mutants had maintained their effectiveness in fixing nitrogen. TAL 379 and TAL 378 on *Glycine max,* TAL 309 on *Vigna unguiculata* and TAL 620 on *Cicer arietinum* retained their effectiveness. It is concluded that these strains would be useful in identification techniques.

Antisera production: Antisera for strains held in the NifTAL collection are valuable for identifying *Rhizobium* strains used in inoculation response experiments and training course demonstrations. Nine antisera were added this year to make a total of thirty-two antisera in the NifTAL collection.

Persistence and competitiveness: Two criteria used in selecting effective strains of *Rhizobium* are persistence in the soil and competitiveness of the selected strain against other rhizobia in the soil.

An experiment to assess the persistence of two proven-effective strains of *Rhizobium leguminosarum* was conducted in an Ustic Humitropept. Uninoculated *Lens esculenta* seeds were planted into field plots that had been planted the previous year with *Lens esculenta* inoculated with the strains NZP5400 and Hawaii 5-0. As both strains accounted for 80-84% of the strains recovered in the nodules, they were deemed to be persistent in the soil.

The competitiveness of three effective strains of *Rhizobium leguminosarum* was observed in a field experiment. *Lens esculenta* seeds were pelleted with equal numbers of all three strains and planted in plots employing low, medium, and high levels of phosphorus. At all three phosphorus levels the strain Hawaii 5-0 was superior to NZP5400 and Nitragin 128A12. It is assumed that Hawaii 5-0 could be effective on *Lens esculenta* in tropical soils where native *Rhizobium* strains are present at different levels of phosphorus availability.

Strain identification technique: An indirect enzyme-linked immunosorbent assay was developed to identify strains of *Rhizobium* in culture and in nodules of *Lens esculenta*. The method can be used on cells of either fresh or frozen nodules from plants grown in the field or in a growth chamber. This technique can be used for field studies and requires less antisera than other serological techniques.

Catalog of *Rhizobium* strains: A catalog of selected strains from the NifTAL *Rhizobium* Collection was revised and published in September, 1978. Distribution was started in October, 1978, and to date 379 copies have been distributed to scientists in 48 countries.

Field inoculation response: Once the potential effectiveness of a *Rhizobium* strain has been demonstrated, it is important to examine the strain's ability to fix nitrogen under field conditions. Also, since the availability of nutrient elements in the soil affects the nitrogen fixing ability of the rhizobia, it is of value to observe the bacteria's performance under different nutrient regimes.

Three inoculation treatments: (1) inoculated with a mixed inoculum; (2) uninoculated; and (3) uninoculated plus nitrogen were applied over two nutrient levels to *Phaseolus vulgaris* seeds sown in a Humoxic Tropohumult at the HAES Kuiaha site, Maui, Hawaii. Upon visual inspection, the inoculation effected a response at both nutrient levels when compared to the uninoculated (no nitrogen added) treatment. Yield data is not yet available.

Inoculation Methodology

Temperatures: High temperatures have been shown to adversely affect the population of inoculum rhizobia during storage, transport and field handling. Knowledge of critical temperatures for survival of rhizobia could be very valuable in the production and distribution of inoculants throughout the tropics.

Peat based inoculants with ten strains of *Rhizobium* from seven hosts were incubated at 28° C, 37° C, and 46° C. At 28° C and 37° C most strains multiplied satisfactorily and increased from 2×10^8 viable rhizobia per gram to 1×10^{10} viable rhizobia

over a two week period. At 26° C, however, there were no viable rhizobia in the peat inoculants after one week of incubation. The most suitable temperature for growth and survival of these rhizobia during short-term storage was 28° C.

To analyze the effect of short-term exposure to high temperatures, samples of single strain, sterile peat-based inocula of ten *Rhizobium* strains were sent to 70 cooperators throughout the tropics. Each inoculant was packaged with a thermometer to record the highest temperature encountered enroute to its destination. Results are only partial at this date, but initial returns indicate certain rhizobia could survive short exposure to temperatures of up to 40° C without suffering a reduction in the number of bacteria to a level below that recommended for inoculation.

Low cost germplasm storage: It is acknowledged that lyophilization of *Rhizobium* cultures and storage in sealed glass ampoules is the most satisfactory long-term method of maintaining viability of the rhizobia. However, this method is not considered economically practical for many situations. The storage of desiccated rhizobia on porcelain beads offers a low cost alternative, as does storage of cultures on materials which are locally available and not detrimental to the survival and effectiveness of rhizobia.

Materials including Hengar granules, porcelain beads, boiling chips, volcanic cinders and crushed lava rock were used to store desiccated rhizobia for one year. Only the boiling chips proved unsatisfactory in maintaining viability. The long-term viability of rhizobia on porcelain beads was examined by attempting to regenerate 72 strains after 6.5 years of storage. To date only 19% failed to grow after adding a single bead to a yeast-mannitol broth.

In an experiment designed to analyze the effect of storage media on the ability of five *Rhizobium* strains to fix nitrogen effectively in association with four legume species, lyophilized ampoules proved to be the most effective means of storage. No storage method reduced the ability of any strain to increase dry matter production above the uninoculated control. It was concluded that these low cost methods of culture storage could prove satisfactory in those situations where minimum funds are available but where maintaining rhizobia viability and effectiveness are important.

Inoculation rates: The inoculant rhizobia compete with the *Rhizobium* strains present in the soil for nodule sites on the host legume. This competition is especially important where the naturally-occurring rhizobia are equally infective but less effective in fixing nitrogen than the introduced inoculum strain.

Three single-strain inocula were applied at five rates each on *Vigna unguiculata* seeds planted in a Hydric Dystrandept which had a rhizobia population of $1.1/10^6$/gram soil. The inoculation rate affected which strains were recovered in the nodules.

At rates of greater than 10^5 rhizobia per seed, more than 50% of the sampled nodules had been infected by the inocula strains whereas when inoculation rates of 10^8 per seed were used virtually all the sampled nodules had been infected by the introduced strains.

Maximizing Nitrogen Fixation through Management

Starter nitrogen effects: The presence of nitrate and ammonium ions in the rhizosphere of the legume plants inhibits biological nitrogen fixation. However, a small amount of "starter" fertilizer nitrogen is known to increase the yields of legumes.

An experiment was carried out to seek an explanation for this starter-nitrogen effect. Four rates of nitrate-nitrogen were applied to two varieties of *Glycine max* and one variety of *Vigna unguiculata* after the seeds had been inoculated with effective *Rhizobium* strains and nodules had developed (10-14 days). The nitrate treatments were applied and withdrawn at different times during the plants' growth to measure the effect of supplementary nitrogen on nodule activity and to assess the rapidity with which nodule activity was resumed once the nitrate source was removed.

The addition of nitrate-nitrogen suppressed nitrogen fixation and increased the rate of plant growth. Once the nitrate supply was discontinued the nodule activity resumed, and the bacteria fixed more nitrogen than the untreated control. For example, within four days after removal of the nitrate treatment, specific activity was 50% higher than the control, the acetylene reduction was the same as the control and nodule mass had more than doubled.

It is felt that the additional nitrogen caused a larger plant, which was later able to supply a greater quantity of photosynthate for nodule activity once the supplemental nitrate was discontinued. The potential benefit of the starter nitrogen on plant growth and symbiotic nitrogen fixation will be investigated under field conditions.

Phosphorus nutrition: Phosphorus deficiency, common in tropical soils, may severely limit both the formation of nodules and the fixation of nitrogen by rhizobia. Some tropical legume species are able to overcome this soil nutrient deficiency by their adapted ability to extract or mobilize phosphorus.

There is some concern that symbiotic nitrogen fixation may have an effect on the phosphorus requirement of the host plant and on the ability of the plant to offset a soil phosphorus deficiency. The phosphorus requirements of well-nodulated *Glycine max* and *Vigna unguiculata* were compared to poorly-

nodulated plants of the same species grown in Humoxic Tropohumult at the HAES Kuiaha site in Hawaii.

Vigna unguiculata was more tolerant of low soil phosphorus levels than soybeans, especially when well nodulated. The external phosphorus requirement of the well-nodulated *Glycine max* was 50-75 percent greater than that of the poorly-nodulated *Glycine max*. No comparable difference in phosphorus requirement was found between well-nodulated and poorly-nodulated *Vigna unguiculata*. It is concluded that some grain legumes (e.g. *Glycine max*) require more phosphorus when well-nodulated than when poorly-nodulated. It would therefore prove beneficial to conduct soil tests for phosphorus availability and assure basal supply for the specific crop to be grown before inoculation and planting.

Training in Rhizobium *Technology*

Nairobi, Kenya: Detailed planning has been carried out to conduct a six-week training course in Nairobi, Kenya. The course is to be held July 30-September 7, 1979 in cooperation with the Kenya MIRCEN (University of Nairobi/UNEP/UNESCO/ICRO). Fifteen persons from Africa and the Middle East, chosen on the basis of past training and future involvement in symbiotic nitrogen fixation, were invited to participate as trainees in this course taught by NifTAL Project staff and regional instructors.

Porto Alegre, Brazil: NifTAL has made arrangements to co-sponsor a three-week course in *Rhizobium* technology in Porto Alegre, Brazil. The course is being hosted by the Brazil MIRCEN (Federal University of Rio Grande do Sul/UNEP/UNESCO/ICRO) and will be presented July 9-July 27, 1979. Dr. Joe C. Burton of the Nitragin Company/Milwaukee will teach one week of the course as a NifTAL funded consultant.

Intern training: An intern training program has been initiated to offer practical training to key personnel involved in *Rhizobium* research or inoculant production in countries throughout the tropics. The trainees are invited to spend two to six months at the NifTAL site where they will follow the NifTAL training course outline while receiving individual instruction from staff scientists. Dr. Bhaskar Reddy of India recently completed the first intern trainee course at the NifTAL site in Hawaii.

Training manual: A course manual in *Rhizobium* technology has been prepared for use at the 1979 training courses. It is divided into four sections: 1) General *Rhizobium* Microbiology; 2) Identification Techniques; 3) Inoculation Technology; and 4) Field and Greenhouse Experimentation. Nineteen exercises offer detailed instructions on most of the techniques used in

Rhizobium technology. The manual will also serve as a laboratory reference tool in the trainees' home labs.

Network Establishment

In January 1979, NifTAL hosted scientists and research administrators representing six universities and eleven international research programs at a workshop to plan an experimental network. Workshop participants discussed the planning of an international network of experiments for evaluating response of legumes to inoculation.

Two resolutions were passed by the participants. The first endorsed the use of available resources to foster field verification programs. The second recommended "support" by individuals and institutions that can contribute to the objectives of the network.

Administration: Many suggestions were offered regarding the best manner in which to establish and administer the Network. NifTAL was charged by those at the Workshop to take a major promotional responsibility for setting up and coordinating the International Network of Legume Inoculation Trials (INLIT) throughout the tropics.

Experiment design: Preliminary designs for the three types of INLIT network experiments were drawn up during the workshop. The experiments include: 1) a simple field trial to determine response of legumes to inoculation with rhizobia, 2) a glasshouse experiment comparing strains recommended by NifTAL with strains of interest to the local collaborators; and 3) a larger field trial to follow up on the glasshouse experiment. The first field experiment (Experiment A) has been implemented at two sites in Hawaii as a pilot INLIT experiment and to work out any unforeseen problems.

Potential collaborators: A list of over 60 potential collaborators for the network experiments was prepared based on suggestions offered by the participants. These and other potential collaborators are being contacted by NifTAL scientific staff.

Dissemination of Results

NifTAL's staff ensures that NifTAL research findings gain exposure in scientific circles in the United States and throughout the tropics. This is accomplished by submitting articles for publication in scientific journals, presenting papers at scientific meetings, participating in seminars, and through dialogues with visitors to the NifTAL site. Findings are also incorporated into training courses,

after which NifTAL-trained technicians will be able to make practical application of NifTAL research in their home countries. Senior staff members also take advantage of international travel to confer with other scientists about ongoing research at NifTAL.

Additionally, NifTAL's Information Section has made considerable progress in reaching other scientists and research projects. Production of "*The Legume/*Rhizobium *Symbiosis in Tropical Agriculture: A Selective Biography with Annotations*" was completed. This bibliography contains over 1100 references to pertinent literature. Copies were sent out to 256 foreign and 121 U. S. addresses. Since then, 137 requests have been filled and 200 additional copies were distributed by the University of Hawaii College of Tropical Agriculture. Annual supplements will keep this valuable document up to date.

FORECAST

Plan of Work - July 1, 1979 - June 30, 1980

ACTIVITY	SCOPE OF WORK	OUTPUT
NETWORK		
General	Develop links with AID missions to assess local needs and potential collaborating institutions.	AID Mission Contacts. Potential collaborators and sites.
	Develop, produce, and distribute informational materials about the Network.	Brochures. Proceedings.
Asia/ Africa/ Americas	Make contacts with host national scientists through travel and correspondence.	Potential collaborators and sites.
	Select cooperators and experiment sites.	
	Begin INLIT trials.	Sites with inoculation response experiments.
Americas	Arrange BNF Conference in Latin America. Joint NifTAL/CIAT sponsorship.	Conference.

ACTIVITY	SCOPE OF WORK	OUTPUT
TRAINING		
General	Develop, produce, and distribute an informational brochure on the Hawaii based, intern training program in *Rhizobium* technology for LDC nationals who hold key positions in *Rhizobium* research or inoculant production within their country's institutions.	Brochures. Links with potential students. Links with potential collaborating institutions.
	Develop, produce, and implement pilot autotutorial, audio-visual modules for use during intern training.	Self-teaching modules.
	Further develop and refine training manual for use in intern and external courses.	Training manual.
Intern	Train four technicians from LDC's in *Rhizobium* technology at the NifTAL site in Hawaii.	Four trained technicians.
External Courses	Implement training course in Nairobi, Kenya, with host institution University of Nairobi and Nairobi MIRCEN.	Thirteen trained technicians.
	Make organizational arrangements for launching a training course in Latin America during the 1979-80 fiscal year.	Collaborative links.
	Hold a training course in Latin America for approximately 15 technicians from the Latin American region.	Fifteen trained technicians.
Graduate assistantships	Offer grants to five graduate students from LDC's and the United States who are pursuing Masters or Ph.D. degrees with a thesis topic directly related to symbiotic nitrogen fixation by tropical agricultural legumes.	Degree training in Microbiology/Agronomy.

ACTIVITY	SCOPE OF WORK	OUTPUT
GERMPLASM		
Promotion	Develop, produce, and distribute informational brochures on germplasm services available to workers in the tropics.	Brochures.
Collection	Receive *Rhizobium* strains from cooperators in the tropics and incorporate these into existing collection.	Expanded collection.
	Actively collect effective strains for *Leucaena leucocephala* and the underexploited legumes.	
	Actively collect *Rhizobium* strains effective in stress soils (i.e., acidity, salinity, low nutrient element status).	Expanded collection.
Isolation	Isolate rhizobia from plant nodule as a necessary step in active collecting and for cooperating scientists throughout the tropics.	Service.
Characterization	Develop and apply a standardized description for *Rhizobium* strains on slants or plates.	Technology.
Preservation	Revise present culture collection to ensure purity of strains; divide collection into: a "classic" collection of 100 fully authenticated strains stored in lypholized ampoules; and a working collection of all presumptive rhizobia stored on porcelain beads.	Improved collection.
Record-keeping	Computerize all information relevant to the collection for convenient, current access.	Accessible collection.
	Develop, produce, and distribute a catalog showing current information on all strains held in the collection.	Catalog.

ACTIVITY	SCOPE OF WORK	OUTPUT
Strain Selection		
Stage I	Authenticate strains as *Rhizobium* in tubes under growth room conditions.	Authenticated strains.
Stage II	Test authenticated strains for potential nitrogen fixation effectiveness in Leonard jars under greenhouse conditions.	Potentially effective strains.
Stage III	Test potentially effective strains in pots of field-collected soil under greenhouse conditions to examine their effectiveness under simulated field conditions.	Pot-tested potentially effective strains.
Stage IV	Test potentially effective, pot-testedstrains in the field at sites in Hawaii and elsewhere in the tropics to examine strain effectiveness under local soil and climatic conditions.	Field proven collective strains.
Stage V	Test potentially effective, pot-tested strains in the field at sites in Hawaii and elsewhere in the tropics to examine strain effectiveness under local soil and climatic conditions.	Network trials. (INLIT)
Recommendations	Produce a catalog listing recommended strains selected through the above process.	Catalog of recommended strains.
Inoculant Production	Design and construct an inoculant production plant to supply peat-based, legume inoculants (using recommended strains).	Inoculants to scientific cooperators in tropical LDC's.
	Investigate culture broth dilution procedures to simplify the inoculant production systems.	

BUDGET FORECAST

Estimated Expenditures and Obligations

July 1, 1979 - June 30, 1980

According to Inputs:

Salaries/Wages		$265,000
Fringe Benefits		51,000
Consultants		4,000
Network		65,000
Travel/Subsistence		28,000
Participants		30,000
Freight		4,000
Publications		8,000
Equipment/Supplies		62,000
Subcontract		36,000
Vehicle Lease		2,700
Overhead		90,000
TOTAL		$645,700

According to Outputs:

Network	50%	$322,850
Training	25%	161,425
Germplasm	10%	64,570
Inoculant technology	10%	64,570
Delivery technology	5%	32,285
TOTAL		$645,700

STATEMENT OF EXPENDITURES AND OBLIGATIONS
AND CONTRACTOR RESOURCES

I. Expenditures and Obligations by Input.

Category	Amount
Salaries and Wages	$202,500.00
Fringe Benefits	32,000.00
Consultants	4,500.00
Network Costs	15,500.00
Travel & Subsistence	12,000.00
Participant Costs	5,500.00
Freight	-0-
Publication	2,300.00
Equipment & Supplies	89,000.00
Subcontract	35,500.00
Vehicle Lease	3,000.00
Overhead	61,000.00
Grand Total	$462,800.00

II. Estimated Expenditures and Obligations by Output.

Culture Collection	$46,280.00
Selection of Superior Strains	115,700.00
Network of Field Testing	37,024.00
Inoculation Methodology	69,420.00
Legume N Contribution	138,840.00
Training	55,536.00
Total	$462,800.00

APPENDICES

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Fig. 1. Effect of inoculation with three strains of *Rhizobium* on dry weights of plant tops of twelve cultivars of *Arachis hypogaea* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

STRAIN:	TAL 1000			TAL 236			TAL 309		
Rank order	Cultivar	Top wt. of plant tops (g/pot)	% of +N treat-ment	Cultivar	Top wt. of plant tops (g/pot)	% of +N treat-ment	Cultivar	Top wt. of plant tops (g/pot)	% of +N treat-ment
1	Valencia R-2	16.2	145	Early Bunch	14.3	99	Blanco Rio Seg.	9.8	92
2	Giza - 4	17.8	118	Egret	8.4	78	F3 - 261	5.8	88
3	Early Bunch	16.9	117	Col. Correntino	9.0	75	Col. Correntino	8.9	74
4	Sula-Smith	13.0	107	Valencia R-2	8.0	71	Giza - 4	11.0	73
5	F3 - 261	7.0	106	F3 - 261	4.6	68	Egret	7.0	65
6	Tato - 34	13.5	91	Blanco Rio Seg.	6.5	61	156 of Cuba	9.9	63
7	Egret	9.8	91	Tato - 34	8.9	60	Bachimba	8.4	58
8	Blanco Rio Seg.	9.6	91	Bachimba	8.3	57	Sula-Smith	4.7	46
9	Makula Red	9.3	77	Giza - 4	8.0	53	Early Bunch	6.3	44
10	Col. Correntino	8.3	69	Sula-Smith	6.2	48	Valencia R-2	4.7	42
11	Bachimba	9.5	65	Makula Red	5.3	44	Makula Red	4.4	36
12	156 of Cuba	8.9	56	156 of Cuba	3.6	23	Tato - 34	4.5	30

Least Significant Difference (L.S.D.) (p=0.05) = 3.6 g

Fig. 2. Effect of inoculation with twenty-three strains of *Rhizobium* on top dry weights of *Vigna aconitifolia* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 1000	*Arachis hypogaea*	3.24
2	TAL 309	*Dolichos africanus*	3.07
3	TAL 999	*Vigna unguiculata*	2.97
4	TAL 169	*Vigna unguiculata*	2.67
5	TAL 187	*Sphenostylis macrocarpa*	2.14
6	TAL 256	*Cajanus cajan*	2.10
7	TAL 648	*Psophocarpus tetragonolobus*	1.89
8	TAL 199	*Stizolobium deeringianum*	1.87
9	TAL 651	*Calopogonium mucunoides*	1.83
10	TAL 642	*Lablab purpureus*	1.78
11	TAL 608	*Stizolobium deeringianum*	1.65
12	TAL 123	*Macroptilium atropurpureum*	1.53
13	TAL 195	*Glycine wightii*	1.33
14	TAL 645	*Vigna angularis*	1.24
15	TAL 766	*Phaseolus acutifolius*	1.11
16	TAL 360	*Vigna luteola*	0.78
17	TAL 22	*Phaseolus lunatus*	0.55
18	TAL 82	*Leucaena leucocephala*	0.14
19	TAL 12	*Sphenostylis macrocarpa*	0.14
20	TAL 379	*Glycine max*	0.14
21	TAL 620	*Cicer arietinum*	0.13
22	TAL 182	*Phaseolus vulgaris*	0.12
23	TAL 113	*Phaseolus* sp.	0.12
	Uninoculated		0.16
	Uninoculated plus N		0.88

Least Significant Difference (p=0.05) = 0.49 g

*Mean of three pots with two plants per pot.

Fig. 3. Effect of inoculation with twenty-three strains of *Rhizobium* on top dry weights of *Glycine wightii* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 309	*Dolichos africanus*	1.14
2	TAL 684	*Vigna frutescens*	0.94
3	TAL 163	*Vigna unguiculata*	0.94
4	TAL 169	*Vigna unguiculata*	0.77
5	TAL 175	*Arachis hypogaea*	0.73
6	TAL 195	*Glycine wightii*	0.63
7	TAL 187	*Sphenostylis stenocarpa*	0.54
8	TAL 648	*Psophocarpus tetragonolobus*	0.50
9	TAL 437	*Vigna radiata*	0.50
10	TAL 734	*Crotolaria juncea*	0.45
11	TAL 22	*Phaseolus lunatus*	0.41
12	TAL 999	*Vigna unguiculata*	0.40
13	TAL 822	*Desmodium uncinatum*	0.37
14	TAL 656	*Pachyrrhizus erosus*	0.35
15	TAL 1000	*Arachis hypogaea*	0.28
16	TAL 691	*Desmodium* sp.	0.22
17	TAL 643	*Canavalia gladiata*	0.22
18	TAL 82	*Leucaena leucocephala*	0.08
19	TAL 832	*Pueraria phaseoloides*	0.06
20	TAL 651	*Calapogonium mucunoides*	0.06
21	TAL 379	*Glycine max*	0.05
22	TAL 276	*Desmanthus virgatus*	0.05
23	TAL 254	*Indigofera endecaphylla*	0.05
	Uninoculated		0.04
	Uninoculated plus N		2.01

Least Significant Difference (p=0.05) = 0.29 g

*Mean of three pots with two plants per pot.

Fig. 4. Effect of inoculation with twenty-two strains of *Rhizobium* on top dry weights of *Vigna umbellata* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 22	*Phaseolus lunatus*	2.25
2	TAL 768	*Vigna angularis*	1.90
3	TAL 656	*Pachyrrhizus erosus*	1.90
4	TAL 643	*Canavalia gladiata*	1.82
5	TAL 169	*Vigna unguiculata*	1.66
6	TAL 607	*Crotolaria usarmoensis*	1.65
7	TAL 648	*Psophocarpus tetragonolobus*	1.52
8	TAL 360	*Vigna luteola*	1.48
9	TAL 608	*Stizolobium deeringianum*	1.46
10	TAL 19	*Voandzeia subterranea*	1.42
11	TAL 999	*Vigna unguiculata*	1.42
12	TAL 651	*Calopogonium mucunoides*	1.34
13	TAL 645	*Vigna angularis*	1.30
14	TAL 766	*Phaseolus acutifolius*	1.27
15	TAL 646	*Cajanus cajan*	1.21
16	TAL 766	*Phaseolus acutifolius*	1.19
17	TAL 780	*Phaseolus radiatus*	0.98
18	TAL 309	*Dolichos africanus*	0.43
19	TAL 1000	*Arachis hypogaea*	0.25
20	TAL 310	*Dolichos biflorus*	0.15
21	TAL 82	*Leucaena* sp.	0.15
22	TAL 767	*Vigna angularis*	0.13
	Uninoculated		0.19
	Uninoculated plus N		2.60

Least Significant Difference (p=0.05) = 0.42 g

*Mean of three pots with two plants per pot.

Fig. 5. Effect of inoculation with twenty-one strains of *Rhizobium* on top dry weights of *Voandzeia subterranea* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 169	*Vigna unguiculata*	2.82
2	12704	*Arachis hypogaea*	2.55
3	TAL 437	*Vigna radiata*	2.54
4	176A22	*Psophocarpus* sp.	2.48
5	TAL 651	*Calopogonium mucunoides*	2.47
6	TAL 371	*Dolichos biflorus*	2.30
7	TAL 19	*Voandzeia* sp.	2.04
8	TAL 43	*Voandzeia* sp.	1.91
9	150Z1	*Stylosanthes*	1.78
10	TAL 642	*Lablab purpureus*	1.63
11	TAL 309	*Dolichos africanus*	1.63
12	#1 Hawaii	*Desmodium* sp.	1.44
13	#3 Hawaii	*Desmodium* sp.	1.16
14	TAL 96	*Arachis hypogaea*	0.98
15	TAL 309	*Dolichos africanus*	0.95
16	TAL 385	*Cicer arietinum*	0.93
17	2611	*Vigna unguiculata*	0.93
18	TAL 97	*Voandzeia* sp.	0.84
19	TAL 403	*Stylosanthes* sp.	0.83
20	TAL 386	*Lupinus* sp.	0.78
21	TAL 464	*Arachis hypogaea*	0.57
	Uninoculated		0.69
	Uninoculated plus N		3.41

Least Significant Difference (p=.05) = 0.498 g

*Mean of three pots with two plants per pot.

Fig. 6. Effect of inoculation with twenty-three strains of *Rhizobium* on top dry weights of *Lablab purpureus* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 1000	*Arachis hypogaea*	3.58
2	TAL 309	*Dolichos africanus*	3.55
3	TAL 203	*Dolichos lablab*	3.16
4	TAL 98	*Vigna unguiculata*	3.12
5	TAL 371	*Dolichos lablab*	2.98
6	TAL 169	*Vigna unguiculata*	2.84
7	TAL 748	*Dolichos lablab*	2.77
8	150Z1	*Stylosanthes* sp.	2.64
9	TAL 672	*Dolichos lablab*	2.56
10	TAL 368	*Dolichos* sp.	2.04
11	TAL 651	*Calopogonium mucunoides*	1.75
12	TAL 209	*Vigna radiata*	1.55
13	TAL 747	*Dolichos lablab*	1.44
14	TAL 236	*Arachis hypogaea*	1.42
15	TAL 642	*Dolichos lablab*	1.42
16	TAL 119	*Psophocarpus tetragonolobus*	1.42
17	TAL 92	*Calopogonium* sp.	1.41
18	TAL 22	*Phaseolus lunatus*	1.40
19	TAL 222	*Dolichos* sp.	1.38
20	TAL 117	*Dolichos lablab*	1.37
21	TAL 641	*Vigna unguiculata*	1.29
22	TAL 16	*Arachis glabarata*	1.10
23	TAL 744	*Dolichos* sp.	1.09
	Uninoculated		1.25
	Uninoculated plus N		4.64

Least Significant Difference (p=0.5) = 0.478 g

*Mean of three pots with two plants per pot.

Fig. 7. Effect of inoculation with twenty-three strains of *Rhizobium* on top dry weights of *Pachyrrhizus erosus* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 744	*Dolichos* sp.	2.62
2	TAL 734	*Crotolaria juncea*	2.46
3	TAL 727	*Calopogonium caeruleum*	2.43
4	TAL 657	*Pachyrrhizus erosus*	2.36
5	TAL 731	*Canavalia* sp.	2.25
6	TAL 651	*Calopogonium mucunoides*	2.25
7	TAL 648	*Psophocarpus tetragonolobus*	2.11
8	TAL 1000	*Arachis hypogaea*	1.83
9	TAL 861	*Indigofera hirsuta*	1.74
10	TAL 22	*Phaseolus lunatus*	1.72
11	TAL 1102	*Lupinus* sp.	1.28
12	TAL 848	*Pithocellobium jiringa*	1.23
13	TAL 794	*Stylosanthes hamata*	1.13
14	TAL 169	*Vigna unguiculata*	0.92
15	TAL 819	*Clitoria laurifolia*	0.57
16	TAL 309	*Dolichos africanus*	0.57
17	TAL 82	*Leucaena leucocephala*	0.57
18	TAL 143	*Voandzeia subterranea*	0.55
19	TAL 187	*Sphenostylis stenocarpa*	0.54
20	TAL 849	*Pithocellobium jiringa*	0.51
21	TAL 656	*Pachyrrhizus erosus*	0.50
22	TAL 182	*Phaseolus vulgaris*	0.47
23	TAL 742	*Desmodium heterophyllum*	0.46
	Uninoculated		0.46
	Uninoculated plus N		3.32

Least Significant Difference (p=0.05) = 0.50 g

*Mean of three pots with two plants per pot.

Fig. 8. Effect of inoculation with twenty-three strains of *Rhizobium* on top dry weights of *Desmodium canum* grown at the greenhouse at Hamakuapoko, Hawaii, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 682	*Desmodium intortum*	0.83
2	TAL 691	*Desmodium* sp.	0.66
3	TAL 282	*Desmodium canum*	0.64
4	TAL 820	*Desmodium ovalifolium*	0.61
5	TAL 666	*Stylosanthes* sp.	0.60
6	TAL 309	*Dolichos africanus*	0.58
7	TAL 999	*Vigna unguiculata*	0.57
8	TAL 281	*Desmodium canum*	0.52
9	TAL 127	*Desmodium uncinatum*	0.51
10	TAL 821	*Desmodium intortum*	0.48
11	TAL 667	*Desmodium intortum*	0.43
12	TAL 569	*Desmodium uncinatum*	0.42
13	TAL 124	*Desmodium intortum*	0.42
14	TAL 186	*Desmodium triflorum*	0.40
15	TAL 169	*Vigna unguiculata*	0.39
16	TAL 284	*Desmodium sandwicense*	0.38
17	TAL 407	*Lupinis mutabalis*	0.35
18	TAL 1000	*Arachis hypogaea*	0.32
19	TAL 822	*Desmodium uncinatum*	0.29
20	TAL 235	*Vigna unguiculata*	0.27
21	TAL 641	*Vigna unguiculata*	0.13
22	TAL 437	*Vigna radiata*	0.12
23	TAL 202	*Desmodium barbatum*	0.06
	Uninoculated		0.08
	Uninoculated plus N		0.95

Least Significant Difference (p=0.05) = 0.53 g

*Mean of three pots with two plants per pot.

Fig. 9. Effect of inoculation with various strains of *Rhizobium* on top dry weights of *Cajanus cajan* grown in Leonard jars at Hamakuapoko, Hawaii, August-September, 1978.

Rank order	Treatment	Host plant	Dry wt. of plant tops (g/pot)*
1	TAL 999	*Vigna unguiculata*	1.49
2	TAL 1039	*Cajanus cajan*	1.22
3	TAL 255	*Cajanus cajan*	1.10
4	TAL 998	*Cajanus cajan*	0.94
5	TAL 979	*Cajanus cajan*	0.89
6	TAL 977	*Cajanus cajan*	0.89
7	TAL 976	*Cajanus cajan*	0.79
8	TAL 579	*Canavalia* sp.	0.64
9	TAL 971	*Cajanus cajan*	0.61
10	TAL 974	*Cajanus cajan*	0.61
11	TAL 970	*Cajanus cajan*	0.55
12	TAL 1075	*Cajanus cajan*	0.55
13	TAL 975	*Cajanus cajan*	0.55
14	TAL 1074	*Cajanus cajan*	0.54
15	TAL 973	*Cajanus cajan*	0.52
16	TAL 1065	*Cajanus cajan*	0.51
17	TAL 1076	*Cajanus cajan*	0.47
18	TAL 981	*Vigna unguiculata*	0.46
19	TAL 980	*Vigna unguiculata*	0.38
20	TAL 972	*Cajanus cajan*	0.38
21	TAL 1120	*Cajanus cajan*	0.26
22	TAL 978	*Cajanus cajan*	0.26
23	TAL 569	*Desmodium uncinatum*	0.25
	Uninoculated		0.27
	Uninoculated plus N		2.20

Least Significant Difference (p=0.05) = 0.24 g

*Mean of three pots with two plants per pot.

Fig. 10. Effect of inoculation with seven *Rhizobium* strains on top dry weights of two cultivars of *Arachis hypogaea* grown in Leonard jars at Hamakuapoko, Hawaii, 1978.

Cultivar:	BURPEE SPANISH			FLORUNNER		
Rank order	Treatment	Dry wt. of plant tops (g/pot)	Range*	Treatment	Dry wt. of plant tops (g/pot)	Range*
1	AH 8	13.6	a	TAL 1000	14.0	a
2	TAL 1000	13.5	a	T-1	12.8	a
3	T-1	11.2	b	AH 8	10.2	b
4	AH 10	7.7	c	AH 10	8.6	bc
5	SU-in	6.0	cd	SU-in	6.8	cd
6	TAL 651	5.8	cd	TAL 301	6.7	cd
7	TAL 301	5.4	d	TAL 651	5.3	d
	Uninoculated	5.3	a	Uninoculated	6.6	a
	Uninoculated Plus N	14.1	d	Uninoculated Plus N	14.7	d

*Means in the same group not followed by the same letter are different at $p<.05$ according to Duncan's New Multiple Range Test.

Fig. 11. Effect of inoculation with six strains of *Rhizobium* on the top dry weights of three cultivars of *Vigna unguiculata* grown in Leonard jars at Hamakuapoko, Hawaii, May, 1979.

Cultivar:	JVU 1190		PARASTOO		CAMARAN	
Rank order	Treatment	Dry wt. of plant tops (g/pot)	Treatment	Dry wt. of plant tops (g/pot)	Treatment	Dry wt. of plant tops (g/pot)
1	TAL 173	4.11	TAL 173	4.10	TAL 999	3.03
2	TAL 999	3.96	TAL 11	3.66	TAL 309	2.89
3	TAL 169	3.83	TAL 999	3.61	TAL 173	2.67
4	TAL 309	2.98	TAL 169	3.59	TAL 11	2.56
5	TAL 11	2.70	TAL 309	3.36	TAL 169	2.51
6	TAL 1073	2.62	TAL 1073	2.70	TAL 1073	2.45
	Uninoc.	0.68	Uninoc.	1.19	Uninoc.	2.95
	Uninoc. plus N	5.98	Uninoc. plus N	5.19	Uninoc. plus N	0.51
Least Significant Difference		0.73 g		0.73 g		0.73 g

Fig. 12. Effect of inoculation with six strains of *Rhizobium* on the top dry weights of three cultivars of *Phaseolus lunatus* grown in the greenhouse at Hamakuapoko, Hawaii, 1979.

Cultivar:	PERU		IITA		BURPEE'S BABY	
Rank order	Treatment	Dry wt. of plant tops (g/pot)	Treatment	Dry wt. of plant tops (g/pot)	Treatment	Dry wt. of plant tops (g/pot)
1	TAL 22	8.21	TAL 22	5.53	TAL 22	7.26
2	TAL 169	5.76	TAL 169	5.10	TAL 169	6.56
3	TAL 642	1.82	TAL 309	1.84	TAL 309	1.84
4	TAL 157	1.76	TAL 157	1.83	TAL 1018	1.65
5	TAL 1018	1.54	TAL 642	1.74	TAL 157	1.54
6	TAL 309	1.43	TAL 1018	1.32	TAL 642	1.50
	Uninoc.	1.92	Uninoc.	1.81	Uninoc.	1.35
	Uninoc. plus N	8.14	Uninoc. plus N	8.89	Uninoc. plus N	7.56

Least Significant Difference (p=0.05) = 1.04g

Fig. 13 Effect of inoculation with various strains of *Rhizobium* on dry weights of plant tops of four cultivars of *Phaseolus vulgaris* grown 34 days in Leonard jars at Hamakuapoko, Hawaii, July-August, 1978.

Cultivar:	MAR MAR		RED HARICOT		CANADIAN WONDER		ZEBRA BEAN	
Rank order	Treatment	Top dry weight (g/pot)	Treatment	Top dry weight (g/pot)	Treatment	Top dry weight (g/pot)	Treatment	Top dry weight (g/pot)
1	TAL 662	2.60	TAL 182	2.38	TAL 182	2.14	TAL 1121	3.36
2	TAL 182	2.43	TAL 241	1.92	TAL 1026	1.93	TAL 182	3.15
3	TAL 664	2.43	TAL 664	1.82	TAL 1028	1.84	TAL 241	2.86
4	TAL 659	2.33	TAL 659	1.77	TAL 661	1.78	TAL 462	2.74
5	TAL 241	2.32	TAL 1121	1.68	TAL 1027	1.77	TAL 664	2.55
6	TAL 462	2.00	TAL 662	1.56	TAL 659	1.75	TAL 659	2.46
7	TAL 460	1.78	TAL 661	1.53	TAL 462	1.75	TAL 783	2.21
8	TAL 660	1.64	TAL 462	1.47	TAL 664	1.65	TAL 661	2.20
9	TAL 783	1.06	TAL 663	1.39	TAL 662	1.49	TAL 663	2.10
10			TAL 660	1.24	TAL 241	1.41	TAL 460	2.09
11			TAL 460	1.18	TAL 660	1.33	TAL 662	2.06
12			TAL 783	0.69	TAL 663	1.29	TAL 660	2.02
13					TAL 460	1.21		
14					TAL 1032	1.08		
15					TAL 783	1.06		
	Uninoc.	1.43	Uninoc.	0.74	Uninoc.	1.05	Uninoc.	1.90
	Uninoc. Plus N	3.71	Uninoc. Plus N	2.83	Uninoc. Plus N	2.59	Uninoc. Plus N	3.79
Least Significant Difference (p=0.05) =		0.54		0.32		0.41		0.42

Fig. 14. Effectiveness of antibiotic-resistant mutant strains compared to parent strains in increasing dry matter (DM) production over uninoculated control at Hamakuapoko, Hawaii, 1979.

	HOST PLANT:	*Glycine max*	*Vigna Unguiculata*	*Cicer Arietinum*
Rank order	Treatment*	Dry wt. (g)	Dry wt. (g)	Dry wt. (g)
1	TAL 379 Spc	8.47		
2	TAL 379 Spc	8.47		
3	TAL 379 Spc	8.25		
4	TAL 379 Spc/Str	8.17		
5	TAL 379 Str	8.12		
6	TAL 379 Parent	7.96		
7	TAL 379 Spc/Str	7.50		
8	TAL 379 Spc/Str	7.43		
9	TAL 378 Spc	7.14		
10	TAL 378 Str	6.68		
11	TAL 378	6.17		
12	Uninoculated	0.92		
	L.S.D.	0.85		
1	TAL 309 Spc		7.05	
2	TAL 309 Str		6.99	
3	TAL 309		6.40	
4	Uninoculated		0.73	
	L.S.D.		1.14	
1	TAL 620 Str			3.34
2	TAL 620			3.32
3	TAL 620 Spc			3.21
4	Uninoculated			0.72
	L.S.D.			0.58

*Spc - Spectinomycin mutant
Str - Streptinomycin mutant
Spc/Str - Spectinomycin/Streptomycin mutant

Fig. 15. Persistence of three strains of *Rhizobium leguminosarum* in an Inceptisol, as measured by percent of nodules formed by these introduced strains one year following introduction in Hawaii, 1978.

Inoculum strains*	Strains recovered in nodules (% of total)			
	NZP 5400	Hawaii 5-0	Both Strains	Unknown
Year 1 (1978) Inoculated				
NZP 5400	100	0	--	0
Hawaii 5-0	0	100	--	0
NZP 5400 + Hawaii 5-0	32	30	38	0
Year 2 (1979) Uninoculated				
NZP 5400	80	4	6	10
Hawaii 5-0	3	84	11	2
NZP 5400 + Hawaii 5-0	19	51	24	6

*10^5 rhizobia (single or mixed) were pelleted onto each lentil seed in 1978. No inoculum was applied in 1979.

Fig. 16. Competition of three introduced strains of *Rhizobium* bacteria in an Oxisol as measured by percentage of nodules formed by the introduced strains. Hawaii, 1978.

Phosphorus level*	Single, double & treble infection of nodules Strains recovered in nodules (% of total)							
	A	B	C	A&B	A&C	B&C	A&B&C	UNKNOWN
Low (.003 ppm)	54	16	5	14	3	8	0	0
Medium (.05 ppm)	48	10	10	14	15	1	0	2
High (0.8 ppm)	60	5	10	17	3	5	0	0

Note: All seeds received a triple inoculum of Hawaii 5-0 (A), NZP 5400 (B), and Nitragin 128A12 (C).

*Seeds were planted in three phosphorus levels. Low = .003 ppm P (Phosphorus in solution); Medium = .05 ppm P; High = .8 ppm P.

Fig. 17. Effect of supplemental nitrogen treatment on symbiotic nitrogen fixation by *Rhizobium* bacteria with the host *Glycine max* grown in the greenhouse at Hamakuapoko, Hawaii, 1978.

Supplemental NO_3 concentration (μM)	Date NO_3 treatment ceased	Dry wt. (grams)	Nodule mass (mg/pl)	Acetylene reduction (μM/pl/hr)	Specific activity (μM/g/hr)
0 (Control)		3.79	355	36.3	101
3	September 1	6.14	545	55.0	106
6	September 6	9.07	475	79.3	167
9	September 8	9.05	220	32.5	148
15	September 12	9.27	98	0.7	10

Fig. 18. Seed or dry matter (DM) yield, relative yield, phosphorus content of index tissue[+], and phosphorus content of harvested *Glycine max* seeds and *Vigna unguiculata* dry matter, as affected by the mode of N nutrition and level of soil P.

PLANT:	*Glycine max*				*Vigna unguiculata*			
Fertilizer P applied (kg/ha)	Seed yield (kg/ha)	Relative yield (% max. yield)	Index tissue P content (%)	Seed P content (%)	DM yield (kg/ha)	Relative yield (% max. yield)	Index tissue P content (%)	DM P content (%)
0 - well nodulated	1030	28	0.22	0.39	4348	72	0.21	0.23
- poorly nodulated	934	19	0.21	0.35	3727	54	0.19	0.22
400 - well nodulated	2699	74	0.37	0.54	5727	94	0.47	0.34
- poorly nodulated	4207	87	0.49	0.61	6592	96	0.50	0.32
620 - well nodulated	3129	86	0.43	0.55	5907	97	0.49	0.35
- poorly nodulated	4468	92	0.49	0.64	6812	99	0.52	0.34
960 - well nodulated	3303	90	0.44	0.55	6025	99	0.49	0.33
- poorly nodulated	4586	95	0.53	0.65	6797	99	0.52	0.34
1360 - well nodulated	3490	96	0.48	0.57	5853	96	0.50	0.34
- poorly nodulated	4836	100	0.52	0.63	6848	100	0.50	0.32
1880 - well nodulated	3638	100	0.50	0.60	6075	100	0.50	0.35
- poorly nodulated	4695	97	0.54	0.65	6830	100	0.52	0.33
L.S.D.$_{.05}$(N)*	210		0.03	0.04	450		n.s.	n.s.
L.S.D.$_{.05}$(N x P)**	305		0.04	0.04	750		0.04	0.05

[+] Index tissue for both legumes was the first fully-expanded leaf, sampled 6 weeks after emergence.

* Least Significant Difference. Used for comparisons between different nodulation levels at the same P level.

** Least Significant Difference. Used for comparisons between different nodulation levels at different P levels or to compare the same nodulation levels at different P levels.

Fig. 19. Distribution of *Rhizobium* cultures by the NifTAL Project. July 1, 1978 - June 30, 1979.

	Country		Number of requests*
	Kenya		3
	U.S.A.		19
	Australia		1
	Trinidad		1
	Thailand		1
	United Kingdom		1
	Mexico		4
	India		2
	India		1
	Saudi Arabia		1
	Korea		1
	Canada		1
	Colombia		1
	Egypt		1
	Indonesia		1
	Spain		1
	France		1
	Puerto Rico		1
Total:	17 countries	Total:	41 requests

* A total of 466 cultures were distributed by NifTAL. All were stored on desiccated beads, with the exception of two deliveries to India, which were peat based.

Fig. 20. Effect of storage methods on the ability of strains to fix N in association with five host species.

Legume/Strain	Rank order	Storage method	Dry weight (grams)
Vigna unguiculata/ TAL 11	1	Lyophilized ampoule	4.01
	2	Black gravel	3.94
	3	Ceramic bead	3.67
	4	Red cinder	3.61
	5	Agar slant	3.33
	6	Uninoculated control	0.88
		L.S.D. (p=0.05)*	0.64
Vigna unguiculata/ TAL 102	1	Lyophilized ampoule	2.95
	2	Ceramic bead	2.16
	3	Ceramic bead	2.03
	4	Uninoculated control	0.46
		L.S.D. (p=0.05)*	0.69
Glycine max/ TAL 102	1	Boiling chip	3.72
	2	Lyophilized ampoule	3.50
	3	Agar slant	3.25
	4	Ceramic bead	3.08
	5	Uninoculated control	2.12
		L.S.D. (p=0.05)*	0.36
Phaseolus lunatus/ TAL 22	1	Lyophilized ampoule	2.47
	2	Boiling chip	2.37
	3	Ceramic bead	2.15
	4	Agar slant	1.51
	5	Uninoculated control	
		L.S.D. (p=0.05)*	
Cajanus cajan/ TAL 155	1	Ceramic bead	0.98
	2	Lyophilized ampoule	0.92
	3	Ceramic bead	0.87
	4	Uninoculated control	0.20
		L.S.D. (p=0.05)*	

*Least Significant Difference

INFORMATION DISSEMINATION

List of Publications sponsored by NifTAL during FY '78-79

Bose, John II. (1979) The legume/*Rhizobium* symbiosis in tropical agriculture: A selective bibliography with annotations. NifTAL Proj. (Univ. of Hi./US Agy for Intnat'l Devel.). 193 pp.

Cassman, Kenneth G. (1979) The phosphorus nutrition of two grain legumes as affected by mode of nitrogen nutrition. PhD Dissertation, Univ. of Hi., Dept. of Agron./Soil Sci. 95 pp.

Keyser, Howard H. and Donald N. Munns. (1979) Effects of calcium, manganese, and aluminum on growth of rhizobia in acid media. Soil Sci. Soc. of Am. Jour. 43(3): 500-503.

Munns, Donald N., et al. (1979) Tolerance of soil acidity in symbiosis of mung bean with rhizobia. Agron. Jour. 71(2): 256-260.

Reyes, Victor G., Padmanabhan Somasegaran, and Donald N. Munns. (1978) Catalog of selected strains from the NifTAL *Rhizobium* collection. NifTAL Proj. (Univ. of Hi./US Agy. for Intnat'l Devel.) 16 l.

Singleton, Paul W. (1979) Effect of inoculation with *Rhizobium* bacteria on *Arachis hypogaea*. M.S. Thesis, Univ. of Hi., Dept. of Agron./Soil Sci. 70 pp.

Woomer, Paul L. (1979) Root tuberization and nitrogen fixation by *Pachyrhizus erosus* (sic). MS Thesis, Univ. of Hi., Dept. of Agron./Soil Sci. 96 pp.

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List of countries receiving NifTAL publications during FY'78-79.

Country	B*	C**	Country	B*	C**	Country	B*	C**
Argentina	4	2	Iran	3	3	Rwanda	3	3
Australia	27	23	Iraq	2	2	Saudi Arabia	3	2
Austria	3	3	Ireland	1	1	Scotland	2	1
Bangladesh	4	4	Israel	3	2	Senegal	5	5
Belgium	1	0	Italy	3	3	Sierra Leone	2	1
Brazil	10	4	Jamaica	1	1	Singapore	1	1
Cameroon	1	1	Japan	9	6	South Africa	5	2
Canada	9	7	Kenya	4	4	Spain	2	2
China	1	1	Lebanon	2	2	Sri Lanka	5	3
Colombia	9	6	Liberia	1	1	Sudan	3	3
Cuba	2	1	Malawi	2	0	Swaziland	1	0
Czechoslovakia	1	1	Malaysia	4	3	Sweden	2	1
Denmark	2	2	Malawi	1	0	Switzerland	1	1
Dominican Republic	3	3	Mauritius	1	1	Taiwan	5	5
Egypt	7	7	Mexico	5	4	Tanzania	6	5
El Salvador	2	2	Nepal	2	1	Thailand	5	4
England	16	11	Netherlands	4	4	Trinidad	4	3
Ethiopia	1	1	New Zealand	10	3	Turkey	2	2
Fiji	1	0	Niger	1	1	Uganda	2	2
France	7	7	Nigeria	13	8	Uruguay	2	0
Germany (W.)	5	3	Norway	1	1	U.S.S.R.	2	0
Ghana	3	3	Pakistan	1	0	Venezuela	5	4
Guadeloupe	1	0	Panama	1	1	Wales	1	0
Guam	1	1	Papua New Guinea	2	2	Western Samoa	1	1
Honduras	1	1	Peru	2	1	Yemen	1	0
India	32	32	Philippines	11	10	Yugoslavia	1	1
Indonesia	3	3	Poland	2	1	Zaire	1	0
						Zambia	1	1

* B= The legume/*Rhizobium* symbiosis in tropical agriculture: a selective bibliography with annotations.
** C= Catalog of selected strains from the NifTAL *Rhizobium* collection, revised September, 1978.

An exchange relationship has been developed with most of the recipients of NifTAL publications, whereby relevant documents are sent to us in exchange for the documents we send. Those recipients in developed countries are, like NifTAL, engaged in research directed toward the alleviation of the world food problem.

Abstracts of publications sponsored by NifTAL during FY '78-79.

*The legume/*Rhizobium *symbiosis in tropical agriculture: A selective bibliography with annotations. J. Bose, II.*

Abstract:

A subject-arranged listing of selected articles appearing mainly in specialized journals from 1971 through mid-1978. Of the 1181 entries, 826 have been annotated to elicit their relevance to the subject of the bibliography. -JB

The phosphorus nutrition of two grain legumes as affected by mode of nitrogen nutrition. K. G. Cassman.

Abstract:

Two sand culture experiments were conducted to examine the effects of phosphorus stress, nodulation and nitrogen source on the growth, dry matter distribution and root development of *Glycine max*. Total dry weight of nitrogen fixing plants grown at optimal phosphorus levels was approximately 60% of that of nitrogen-supplied plants. Mode of nitrogen nutrition had no effect upon the total dry weight of plants grown at deficient phosphorus levels. The root weight: total plant dry weight ratio of nitrogen-fixing plants was less than that of nitrogen-supplied plants. There was an inverse relationship between nodule mass and total root length although the number of first-order lateral roots on nodulated and non-nodulated plants was the same.

A field experiment was conducted to analyze the effect of nitrogen source on the internal and external phosphorus requirements of *Glycine max* and *Vigna unguiculata*. The phosphorus requirements of *Glycine max* were sensitive to the mode of nitrogen supply, whereas those of *Vigna unguiculata* were not. *Glycine max* also exhibited a larger difference in yield potential between the nitrogen-fixing and nitrogen-supplied plants than did *Vigna unguiculata*. -KC

Effects of calcium, manganese, and aluminum on growth of rhizobia in acid media. H. H. Keyser and D. N. Munns.

Abstract:

Growth studies were done in defined liquid media co assess effects of Mn toxicity and Ca deficiency associated with soil acidity. The study included 23 strains of cowpea rhizobia previously found capable of growth at pH 4.5 and 10 strains of *Rhizobium japonicum* tolerant of pH 4.8. The low level of Ca (50 uM) has been found toxic to legume hosts of the strains tested.

In a detailed growth study of three cowpea strains at pH 4.6, low P (10 uM) limited maximum population density in all three strains. Low

Ca limited it in one strain.

A rapid screening method based on attainment of turbidity from a small inoculum was applied to the cowpea rhizobia at pH 4.5 and soybean rhizobia at 4.8. High Mn and low Ca slowed growth of some strains, but Mn stopped growth of none and low Ca stopped growth of only three strains. Neither was as severe a stress as 25-50 uM Al, simultaneously observed and previously reported. All strains tolerant of Al were tolerant of Mn and low Ca.

Possible amelioration of Al toxicity by Ca was tested in three cowpea strains, by a factorial experiment with three Ca levels (50-1,000 uM) and four Al levels (0-100 uM), at pH 4.5 in liquid media. Calcium had a statistically significant protective effect against Al in two strains, but the effects were small and probably of no biological or practical significance.

In acid soils, Al toxicity and acidity itself are probably more important limiters of rhizobial growth than Mn toxicity and Ca deficiency.

Tolerance of soil acidity in symbiosis of mung bean with rhizobia. D. N. Munns, H. H. Keyser, V. W. Fogle, J. S. Hohenberg, T. L. Righetti, D. L. Lauter, M. G. Zaroug, K. L. Clarkin, and K. W. Whitacre.

Abstract:

Variation in tolerance of soil acidity among 40 rhizobial strains was assessed in greenhouse trials in which the strains were applied as separate seed inoculants (5×10^4 cells per seed) to two cultivars of mung bean (*Vigna radiata L.*) and performance was measured by nodulation, growth, and N-yield of the host plant. The plants grew in a low N, low Ca acid subsoil, Goldridge fine sandy loam (Typhic Hapludult, fine loamy, mixed, mesic), left at its natural pH of 5.0 (saturation paste) or limed with $CaCO_3$ to pH 6.3. Each pH x strain treatment was triplicated in separate pots. Strains demonstrated a large and perhaps continuous variation in acid tolerance. A few were very sensitive: they failed to nodulate at pH 5.0. About half were moderately sensitive: nodulation and growth were significantly impaired at pH 5.0. The remainder were tolerant: like NH_4NO_3 they supported similar plant growth at both soil pH values. Some strains combined high tolerance with high effectiveness.

A strain's acid tolerance could not be predicted from the abundance or effectiveness with which it nodulated at favorable pH, or from its growth rate or acid production in conventional yeast mannitol medium.

A few strains were sensitive on one host cultivar and tolerant on the other, implying that acid tolerances of symbiotic legumes cannot be compared validly in trials with only one inoculant.

Catalog of selected strains from the NifTAL Rhizobium *collection.*
V. G. Reyes, P. Somasegaran, and D. N. Munns.

Abstract:

Lists 125 *Rhizobium* cultures from the NifTAL collection which have been recommended for further testing. Preliminary evaluations are based on Leonard jar and/or pot trials. Some include data on tolerance to acid soil stress. The strains are cross-referenced to 31 legumes of tropical importance. Pure cultures of the cataloged strains of *Rhizobium* are available from NifTAL to research workers and inoculum producers. - JB

The symbiotic efficiency of some peanut cultivars and their interactions with strains of Rhizobium Spp. *P. W. Singleton.*

Abstract:

Various strains of *Rhizobium* were used to inoculate four cultivars of *Arachis hypogaea*. Significant differences in strain effectiveness and strain-by-cultivar interactions were observed. A local isolate proved to be the most effective strain.

A growth room study was undertaken to determine if three cultivars of *Arachis hypogaea* varied in time-to-nodulation when inoculated with ten strains of *Rhizobium*. Differences were found between the three cultivars. Time to nodule formation was positively correlated with seed size. Differences in time to nodule formation and strain-by-cultivar interactions were observed. - PS

Root tuberization and nitrogen fixation by Pachyrhizus erosus.
P. L. Woomer.

Abstract:

Pachyrhizus erosus has been cited as an underexplcited legume with potential benefits as a food crop in the developing countries of the tropics. This species has the ability to form symbiotic relationships with nitrogen-fixing *Rhizobium* bacteria thus alleviating the need for high cost, synthetic nitrogen fertilizers.

1) Three treatments: a) inoculated, b) uninoculated plus nitrogen fertilizer, and c) uninoculated/no nitrogen fertilizer added were applied to *P. erosus*, and dry weights, yield components, and percent nitrogen were compared among these treatments. 2) Diurnal fluctuation of nitrogen fixation at different growth stages of *P. erosus* was evaluated by measuring acetylene reduction.
3) Seasonal fluctuation of dry matter and nitrogen distribution

in the plant, and the effect of pod removal on root tuberization were also evaluated.

Only two of the twenty-three strains examined were able to establish highly effective symbiotic relationships with *P. erosus*, when compared to the nitrogen-supplied control. The percent nitrogen in the tissue of the tuberous root was affected by the form of available nitrogen and the nitrogen-fixing effectiveness of the rhizobial strain. Diurnal changes in nitrogenase activity were affected by fluctuations in the environment, but not by changes in root morphology. Roots and seeds were equal, competitive sinks for carbon, but the reproductive structures assimilated more nitrogen than did the tuberous roots. The marketable yield of the roots after 15 weeks' growth was increased 30% by periodic flower removal. - PW

List of meetings attended by NifTAL staff members during FY 1978-79.

1. July 1978. International Legume Conference. Richmond, England.

2. October 1978. IX Latin American Conference on *Rhizobium*.

3. November 1978. American Society of Agronomy Meetings. Chicago, Illinois. Papers presented by Kenneth Cassman and Paul Singleton.

4. January 1979. NifTAL Network Planning Workshop. Kahului, Maui, Hawaii.

5. April 1979. 12th Hawaii Fertilizer Conference. Kahului, Maui, Hawaii. Paper presented by Dr. A. S. Whitney.

6. May 1979. American Society of Microbiologists Meeting. Honolulu, Hawaii. Paper presented by Dr. Jake Halliday.

7. June 1979. North American *Rhizobium* Meetings. College Station, Texas. Papers presented by Dr. Jake Halliday, Dr.Padmanabhan Somasegaran, Sheila May, and Paul Woomer.

Abstracts of papers presented by NifTAL staff members during FY '78 - 79.

The effect of phosphorus stress and nitrogen nutrition upon the growth and root development of nodulated soybean. K. G. Cassman and A. S. Whitney. Presented at American Society of Agronomy Meetings, 1978, Illinois.

Abstract:

A recycling nutrient solution/sand culture system was used to compare the effects of P stress and N source upon the growth, N_2-fixation, and root development of soybean (*Glycine max* (L.) Merr.). Nutrient solution P concentrations and pH were adjusted daily. P treatments included: .005, .02, .05, 0.2, 0.5, and 2.0ppm PO_4-P. Nutrient solution N levels were adjusted weekly. N treatments included: 0, 1.25, 2.5, and 5.0mM N. Seeds were inoculated with 10^7 rhizobia/seed. Plants were harvested 35 to 47 days after planting. Growth of plants in the minus-N nutrient solution at the highest P level (2.0ppm PO_4-P) was half that of plants provided 5.0mM N. At the four lowest P levels, N provided in the nutrient solution did not increase dry weight of plants. There were significant differences in dry matter distributions caused by P stress and N source. At all P levels, plants relying upon symbiotically produced N as their sole N source had a smaller root % of total plant weight than plants provided N. If this phenomenon occurs under field conditions, nodulated soybeans, grown on N-deficient soils may be more susceptible to nutrient stress than their N fertilized counterparts.

Greenhouse studies on the symbiotic effectiveness of 11 tropical peanut cultivars. P. W. Singleton, W. G. Sanford, and K, R. Stockinger. Presented at the American Society of Agronomy Meetings, 1978, Illinois.

Abstract:

A greenhouse experiment was conducted to compare the N_2-fixing capacities of 11 cultivars of peanut *(Arachis hypogaea L.)*. One liter plastic pots containing vermiculite and perlite and connected to a subirrigation system were used to test the effect of four treatments on each cultivar. Treatments consisted of inoculation with three strains of *Rhizobium* spp. and an NH_4NO_3 control. All three strains of *Rhizobium* had previously been shown to be highly effective with two cultivars of peanut. Dry weight data from five week old plants indicate that the cultivars were relatively more variable in symbiotic capacity than when given NH_4NO_3. Cultivar means composed of the three *Rhizobium* treatments (symbiotic mean) ranged from 6.3g/pot to 12.5g/pot. Means of the same cultivars given mineral N ranged from 10.6g/pot to 15.8g/pot. The sumbiotic means of cultivars relative to their respective yield with NH_4NO_3 ranged from .47 to .87. Data for total shoot N showed the same trend. Significant cultivar-by-*Rhizobium* strain interactions were also found.

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Improving the nitrogen contribution of legumes to Hawaii agriculture. A. S. Whitney and Jake Halliday. Presented at the 12th Hawaii Fertilizer Conference, 1979, Kahului, Hawaii.

Abstract:

The goal of the NifTAL Project is to carry out research, training, and service functions which will contribute to the more effective utilization of the ability of selected strains of *Rhizobium* bacteria to symbiotically fix nitrogen for agricultural, tropical legumes.

Strains have been selected at NifTAL for ten major grain legumes, six - eight minor legumes, several miscellaneous legumes, and three pasture legumes. The project is also screening strains for tolerance to acid, infertile soils.

NifTAL has trained 24 technicians from less developed countries through a six-week practical course in *Rhizobium* technology. The course is being presented this year in Nairobi, Kenya. NifTAL has also facilitated the graduate studies of six students who have received Master of Science and Ph.D. degrees.

NifTAL has coordinated a network of cooperators in the tropics to examine the response of legumes to inoculation and select recommended strains for legumes of interest to the cooperators. This initial response to the proposed program has been excellent.

The agronomic research of the NifTAL Project has been oriented toward obtaining a better understanding of those principles that govern nitrogen fixation by tropical legumes. Attention has been given to the effects of lime, phosphorus levels, nitrogen levels, shading and intercropping on yield and nitrogen fixation.

The role of the Rhizobium *germplasm at NifTAL in exploiting the legume/* Rhizobium *symbiosis in the tropics. J. Halliday. Presented at the American Society of Microbiologists Meeting, 1979, Honolulu, Hawaii.*

Abstract:

The first step in NifTAL's strategy to improve the use of legumes in the tropics was to collect strains of *Rhizobium* and assemble a germplasm resource from which strains highly effective in fixing nitrogen through symbiotic association with particular tropical legumes could be selected. NifTAL's collection of over 2000 strains will be organized to fulfill the following roles:

A. Provide long-term storage of authenticated strains for which legume or origin is known and the potential nitrogen fixation effectiveness on at least one tropical legume has been verified. This resource will be maintained in lyophilized, evacuated ampoules at 4° C.

B. Provide for medium-term storage of a large number of presumption strains of *Rhizobium* from a range of tropical legumes and representing a diverse array of geographic regions. This resource will be conserved by the desiccated bead method at 4°C. Services offered by the *Rhizobium* Germplasm Resource at NifTAL are:

1. Isolation and preliminary characterization of presumptive Rhizobia.

2. Supplying of pure strain cultures.

3. Supplying peat-bead inoculants.

4. Safekeeping of replicates of the entire collections of other individuals/institutions as an insurance against catastrophic loss.

The Rhizobium *affinities of* Pachyrhizus erosus (L.). *P. Woomer and P. Somasegaran. Presented at the North American* Rhizobium *Meetings, 1979, Texas.*

Abstract:

The National Academy of Science called attention to the Mexican yam bean *(P. erosus)* as an "under-exploited" legume. Recommendations for cultivation of this tuberous root crop include fertilization with nitrogen, suggesting ignorance of, or inadequacy of, the nitrogen contribution from this legume's association with *Rhizobium*. Twenty-three strains of *Rhizobium* of widely differing origins were used to inoculate *P. erosus* (TPe-1 from IITA, Nigeria). Growth of inoculated plants in Leonard jar culture was compared to uninoculated plants receiving no combined nitrogen and uninoculated plants receiving combined nitrogen (70 ppm N as KNO_3) in the rooting medium. *P. erosus* was nodulated by 20 out of the 23 strains of *Rhizobium* but formed highly effective symbiotic associations with only two strains. The best strains had been isolated originally from *Crotolaria juncea* and *Calopogonium caeruleum*. Strains from *Arachis hypogaea* and *Pachyrhizus tuberosus* also proved moderately effective. The results suggest that there is a potential to increase field performance of *P. erosus* through inoculation with superior strains of *Rhizobium* at the time of sowing. The best strain (TAL 734) produced 80% of the dry matter observed in the combined nitrogen treatment. Partitioning of dry matter between the root and shoot was not affected by strain of *Rhizobium* nor source of nitrogen (symbiotic vs combined). The most effective strain increased the nitrogen content of the tuberous root three-fold over the uninoculated control and in the case of an ineffective strain (TAL 742) the nitrogen content was actually below that of the control (0.28% vs 0.52% N).

The effect of high temperatures on the growth and short-term storage of tropical rhizobia in sterile peat. P. Somasegaran and H. Hoben. Presented at the North American Rhizobium *Meetings, 1979, Texas.*

Abstract:

The ability of tropical rhizobia to grow and maintain high viable populations during storage is an important consideration in the production of peat inoculants. Unfavorable temperatures during transport, distribution and field handling, can affect inoculant quality. Ten strains of *Rhizobium* from *Arachis hypogaea, Cicer arietinum, Glycine max, Leucaena leucocephala, Macroptyloma africanum, Vigna radiata,* and *Vigna unguiculata* were incubated at 28° C, 37° C, and 46° C following inoculation into sterile peat. Most strains multiplied satisfactorily at both 28° C and 37° C attaining populations of more than 1.0×10^{10} viable rhizobia/g peat in the first two weeks (the initial population density was 2×10^{8} rhizobia/g). At 46° C, however, no viable rhizobia could be detected in the peat inoculants after one week's incubation. The most suitable temperature for growth and survival of tropical rhizobia during short-term storage was 28° C. The rate of decline in the population of *Rhizobium* during storage was greater at 37° C than at 28° C. The Miles and Misra drop-plate method, used routinely in this study for counting rhizobia, proved economical of both time and materials and can be recommended for similar experiments in which the carrier for *Rhizobium* is sterile.

The Rhizobium *affinities of some under-exploited legumes. P. Somasegaran. Presented at the North American* Rhizobium *Meetings, 1979, Texas.*

Abstract:

Besides having untapped potentials for food and other products of economic value, the so called "under-exploited" legumes may have high nitrogen-fixing capabilities. These legumes presently occupy restricted regions in certain parts of the tropics and have been virtually unknown as economic crops outside these areas. Therefore introduction of these legumes to new areas within the tropics may bring benefits and lead to a wider exploitation of their potential. Introduction of these legumes without at the same time assuring availability of a fully effective and compatible strain of *Rhizobium* could result in sub-optimal nitrogen fixation. The under-exploited legumes, *Lablab purpureus, Phaseolus lunatus, Phaseolus acutifolius, Voandazeia subterranea, Vigna aconitifolia, Vigna umbellata,* and *Pachyrhizus erosus* were cultured in Leonard jars and inoculated with strains of *Rhizobium* isolated from a diverse array of legume genera and also commonly used commercial strains. Wide variation in effectiveness of the symbiosis with different strains of *Rhizobium* was observed for each legume. *Phaseolus lunatus, Phaseolus acutifolius* and *Pachyrhizus erosus* indicated moderate specificity in that they did not nodulate effectively with rhizobia from most of the legumes of the cowpea group and with broad-spectrum commercial

strains. It is inferred that these moderately specific legumes would benefit from inoculation with *Rhizobium* when introduced into new environments.

Mixed-infection in lentil nodules and its relationship to competitiveness of Rhizobium *strains. S.N. May and B.B. Bohlool. Presented at the North American* Rhizobium *Meetings, 1979, Texas.*

Abstract:

Three highly effective and serologically distinct strains of lentil rhizobia have been tested for their ability to compete for nodule sites on lentils *(Lens esculenta)*. In two separate growth chamber studies, double-strain inocula were applied to lentil seedlings. The first study involved only one cultivar of lentil; the second involved three *(Benewah, Tekoa,* and *Chilean)*. In both these studies, typing of nodules by immunofluorescence revealed a close relationship between competition and the occurrence of doubly-infected nodules. Results of two-strain competition were: NZP 5400 and Hawaii 5-0 were equally competitive, and 30% of the nodules contained both strains. NZP 5400 was superior against Nitragin 128A12, and 6% of the nodules contained both strains. Hawaii 5-0 and Nitragin 128A12 varied in competitiveness depending on the cultivar. When equally competitive, a large percentage of nodules contained both strains, but when only one was superior, no nodules contained both strains. Double-inoculation with NZP 5400 and Hawaii 5-0 in a field trial gave similar results--the two were equally competitive against one another and a high number (over 30%) of double-infections occurred. A persistence experiment in the same plots one year later showed Hawaii 5-0 slightly superior to NZP 5400 and 24% of the nodules contained both strains.

List of visitors to NifTAL Project from July 1, 1978 - June 30, 1979.

NAME	INSTITUTION
Lloyd Frederick	DSB/AGR, AID (Washington, D.C.)
Samson C. S. Tsou	AVRDC
John G. Torrey	Cabot Foundation, Harvard
S. P. R. Weerasinghe	Regional Agri. Research Office Bandaiawela, Sri Lanka
S. O. Keya	University of Nairobi
Fernando Riveros	FAO, Rome
Maurice Fried	FAO/IAEA Joint Division, Vienna
Jake Halliday	CIAT, Cali Columbia
R. Stewart Smith	University of Illinois
William Judy	INTSOY, Puerto Rico
Peter J. Dart	ICRISAT, India
Saleem Ahmed	East-West Center
Gerry Elkan	North Carolina State University
Doug Gross	North Carolina State University
Foster Cady	Cornell University
Martin Alexander	Cornell University
Joe Burton	Nitragin Company
Harrison Brown	East-West Center
Ron Stryker	Raleigh, North Carolina
Joe Salvo	ECOC, Honolulu
Karel R. Schubert	Dept. of Biochemistry, Michigan State
Carlos Ruggiero	Dept. Fitotechnia, Fac. Cienc. Agrarias e Veterinarias, São Paulo, Brazil

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NAME	INSTITUTION
Mercedes Umali-Garcia	U. O. Los Baños, College
Ed Pantstico	Crops Division, PCARR, Los Baños, Philippines
Paul Knowles	University of California, Davis
G. O. Mott	University of Florida
Jerri Lay	Maui Committee: World Hunger Project
Allan Jones	HSPA
Laura de Vasconcelos	Rutgers University - Cook College Biochemistry & Microbiology
S. O. Kan	Dept. of Tropical Medicine University of Hawaii - Manoa
Warren Silver	USDA-SEA/CR
Frank B. Dayzo	Dept. of Microbiology & Public Health Michigan State University
Benjamin H. Beard	USDA-SEA, Agronomy Range Science, University of California, Davis
Enoch M. Ntokotha	Ministry of Agriculture, Lilongawe, Malawi
David J. Harris	University of Hawaii, College of Tropical Agric. & Human Resources
G. Bhaskar Reddy	University of Georgia
Bob Rodelius	Bahama Is.
Chauncey Chong	University of Nevada
Don Plucknett	DSB/AGR, AID

List of University of Hawaii Personnel Visiting NifTAL Headquarters from July 1, 1978 - June 30, 1979.

NAMES	INSTITUTION
Roger Ernst	University of Hawaii College of Tropical Agriculture & Human Resources
Russell Yost	University of Hawaii, College of Tropical Agriculture & Human Res.
James L. Brewbaker	University of Hawaii, College of Tropical Agriculture & Human Res.
Peter P. Rotar	University of Hawaii, College of Tropical Agriculture & Human Res.
James L. Walker	University of Hawaii, College of Tropical Agriculture & Human Res.
William R. Furtick	University of Hawaii, College of Tropical Agriculture & Human Res.
Shelly M. Mark	University of Hawaii, College of Tropical Agriculture & Human Res.
Walter T. Harada	HAES
Taka Izumi	University of Hawaii, College of Tropical Agriculture & Human Res.
Saikon Taal	University of Hawaii, College of Tropical Agriculture & Human Res.
Jim Silva	University of Hawaii, College of Tropical Agriculture & Human Res.
Noel P. Kefford	HAES