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NifTAL Project

June 1, 1977 - June 30, 1978

NifTAL

Maximizing Symbiotic Fixation of Nitrogen

by Grain and Forage Legumes in the Tropics

UH/AID Contract ta-C-1207

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

ANNUAL REPORT

1977 - 1978

NifTAL PROJECT

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

UH/AID Contract No. ta-C-1207

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STATISTICAL SUMMARY

Project Title:	Maximizing Symbiotic Fixation of Nitrogen by Grain and Forage Legumes in the Tropics (NifTAL Project)
Contract No.:	ta-C-1207
Project Directors:	A. S. Whitney, W. G. Sanford, and B. B. Bohlool
Contractor:	University of Hawaii
ontractor's Address:	Honolulu, Hawaii, 96822
Contract Period:	June 1, 1975 to June 30, 1978*
Reporting Period:	June 1, 1976 to June 30, 1978*
Total Expenditures and Obligations Through Previous Contract Year:	$588,337
Added Expenditures and Obligations For Contract Year Just Completed*:	$363,668
Total Expenditures and Obligations to 06-30-78:	$952,005
Estimated Expenditures for Next Contract Year:	$496,612

*Including one-month extension per Amendment

PROJECT PERSONNEL

Project Director and Principal Investigator:	A. S. Whitney
Associate Project Directors:	W. G. Sanford B. B. Bohlool
Soil Microbiologists:	V. G. Reyes P. Somasegaran
Agronomists:	K. R. Stockinger A. B. Guevarra*
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*Completed postdoctoral appointment December, 1977.

NARRATIVE SUMMARY

During the third year of operations, the NifTAL Project program of work expanded further into research, training, and service activities in support of programs concerned with symbiotic nitrogen fixation in tropical developing countries.

Nearly 250 additional strains were acquired for the NifTAL *Rhizobium* collection. Most were proven strains supplied by laboratories in 9 countries, others were isolated from nodules collected by NifTAL staff in Hawaii and other countries. Over 1000 strains are now held in the active and reserve collections. An abbreviated catalogue of strains which performed well in greenhouse screening trials was published in mid-year (December/January) and revised in April, 1978. Cultures of *Rhizobium* strains were supplied to 22 workers in 17 countries representing all the inhabited continents. Requests for cultures are increasing, and it is expected that this service will become increasingly important in coming years.

Screening of *Rhizobium* strains for effectiveness on target legumes continued at a rapid pace, involving over 18 trials on 17 target legumes, including several trials conducted by Dr. D. N. Munns (U.C.--Davis subcontract) which also evaluated the tolerance of cowpea-type strains to acid soils. In addition, two highly-competitive lentil strains were selected by May and Bohlool (U.H.--Manoa Campus) on the basis of growthroom and field tests.

Additional experiments were conducted at NifTAL to test the effects of root-nodule passage on effectiveness of *Rhizobium* strains (2 out of 3 increased in effectiveness) and to evaluate strain tolerance to high temperatures. Other trials were conducted at the University of California to develop selection criteria for acid-tolerant strains, low-phosphate tolerant strains, and salt-tolerant strains.

Field trials were conducted on the island of Hawaii (Benchmark Project site) and on Maui to test the effectiveness of *Rhizobium* strains in the field. In the first trial, two soybean strains significantly exceeded the control but there were no significant differences among strains. In the latter trial, three out of seven lentil strains performed well.

A field experiment was also conducted to assess techniques for replacing native *Rhizobium* strains with an introduced cowpea strain. Very large inoculum levels were required to produce 29 to 34% of the nodules of inoculated cowpea plants, and there was good evidence that placement of the inoculum near the seed was more effective than band or soil-mix applications.

The contribution of legumes to the N economy of cropping systems was evaluated in field and greenhouse experiments. Most of the evidence suggested that *sequential* cropping systems involving grain legumes and grass better exploited the potential of legumes to provide N to the

system than did *intercropping* systems. Perennial forage-type legumes, however, could be successfully intercropped provided there were no alleleopathic interactions between grass and legume. Leaf materials from a number of different legumes incorporated into the soil on an equal-N basis were identical in their release of N to a following grass crop, regardless of N percentage or degree of mixing with the soil.

The effect of liming was studied in the field, and the effects of fertilization with N and P were studied in the greenhouse. The major responses to liming a Tropohumult were: a) at pH 5.0 to 5.3, the legumes responded primarily to reduced aluminum in the soil; b) above pH 5.3, there was a "starter N" effect due to lime-induced mineralization of the N from the soil.

Soybean plants grown in nutrient solution containing various levels of P and N responded strongly to P and (at the highest levels) to N. The poorer growth of the symbiotic (zero N) plants was associated with reduced root development which may have serious implications regarding the yield potential of symbiotic plants.

The project provided intensive training in *Rhizobium* technology to 13 additional technologists during the third year, including 12 from developing countries. These, together with the 12 persons trained previously, are a substantial addition to the pool of workers in developing countries who are well trained in the techniques for *Rhizobium* research.

Several graduate research assistants also worked on N-fixation by tropical legumes under NifTAL auspices. Four were supported by NifTAL, one by the East-West Center, and one by the Hawaii Agricultural Experiment Station. The latter two completed their thesis work during the year, and substantial parts of their dissertations are expected to be published shortly.

Contacts were maintained with research workers throughout the tropics and elsewhere through germplasm and information exchange, attendance and presentation of papers at international conferences, and by travel in developing countries. A "common-interest" network of potential collaborators has thus been cultivated in preparation for the establishment of a formal network in the next phase of the project.

GENERAL BACKGROUND

NifTAL is an acronym for the University of Hawaii's project to conduct service, research, and training programs concerning Nitrogen Fixation by Tropical Agricultural Legumes. The project was conceived as a means of complementing and supporting legume-oriented programs throughout the tropics. The goal is to promote greater exploitation of the nitrogen-fixation potential of tropical legumes in developing countries and thereby provide a basis for increased production of high-protein foods with minimal dependence on nitrogen fertilizer.

Interest in this approach to food production in developing countries has been given strong impetus by an increasing awareness that nitrogen fertilizers are very energy-intensive in a world where competition for energy supplies is becoming more intense. This creates particularly difficult problems for developing countries who must buy their energy (or N fertilizer) with hard currencies. Fortunately, the short-term outlook is for adequate supplies of N fertilizers and a reasonably favorable ratio of fertilizer to food prices. However, the longer-term outlook is for greater reliance on nitrogen fixation by legumes to provide adequate supplies of high-protein foods and feeds with minimal energy inputs. In both short- and long-terms, many LDC farmers will still be unable to afford or obtain N fertilizers and must depend on biological N fixation, mainly by tropical legumes, to maintain adequate levels of food production.

NifTAL's purposes and objectives are designed to provide a foundation of tested rhizobial germplasm, improved inoculation methodology, a cadre of trained technicians, and a better understanding of the nitrogen contributions of legumes in cropping systems, in order that the potential of tropical legumes can be more fully exploited in developing countries for the benefit of their farmers and consumers.

The project is funded by a contract with the U.S. Agency for International Development which was approved in June, 1975 for a 3-year period; and extended June 30, 1978 for an additional 3-year period ending June 30, 1981.

CONTINUED RELEVANCE OF PROJECT PURPOSES

The goals of the NifTAL Project continue to be increasingly relevant in the light of predictions by various institutions and agencies (e.g. the C.I.A.) that energy demands will exceed production capacity within 15 to 20 years, with consequent price and priority adjustments which will undoubtedly affect the cost-benefit analysis of N fertilizer use. In its 1977 "World food and nutrition study, the potential contributions of research" prepared by the National Research Council of the National Academy of Sciences, a very high priority was given to the need for research in biological nitrogen fixation (BNF). The supporting reports on BNF gave topmost priority to the legume/*Rhizobium* symbiosis, and the specific recommendations reflect the objectives of the NifTAL Project. Both the NSF and the FAO have accorded similar priorities to the kinds of activities in which the NifTAL Project has been engaged.

STATEMENT OF PROJECT PURPOSES

The goal of the NifTAL Project is to reduce the dependence of LDC farmers on nitrogen fertilizer for the production of increased quantities of food. This will be accomplished by assisting *Rhizobium* and legume programs throughout the tropics to more effectively exploit the legume-*Rhizobium* symbiosis. Project purposes are thus:

I. From a comprehensive collection of *Rhizobium* strains, select and characterize strains adapted to tropical conditions for distribution to researchers and inoculum producers.

II. Develop improved techniques and cultural systems for ensuring a) dependable effective nodulation, and b) maximum utilization of legume-fixed N.

III. Train LDC researchers and inoculum producers for applied research and technology in *Rhizobium* bacteriology.

IV. Form a network of collaborators for the purposes of exchanging germplasm and research information and for cooperative identification and testing of *Rhizobium* strains.

ACCOMPLISHMENTS TO DATE

PURPOSE I . Assemble and Document a Comprehensive *Rhizobium* Collection.

A. Working Objectives

1) To build a comprehensive collection of *Rhizobium* strains which are known to be effective on legumes important to the tropics.

2) To document as completely as possible the strains in the collection and to enter the information for strains of interest in the Data Retrieval Bank of the World Data Center for Microorganisms (University of Queensland, WFCC, UNESCO and ICRO).

3) To publish a catalogue of the *Rhizobium* strains held by NifTAL--and if possible, the strains held in other collections which are relevant to tropical legumes.

4) To disseminate *Rhizobium* strains to qualified workers for research and inoculant production.

B. Progress to Date

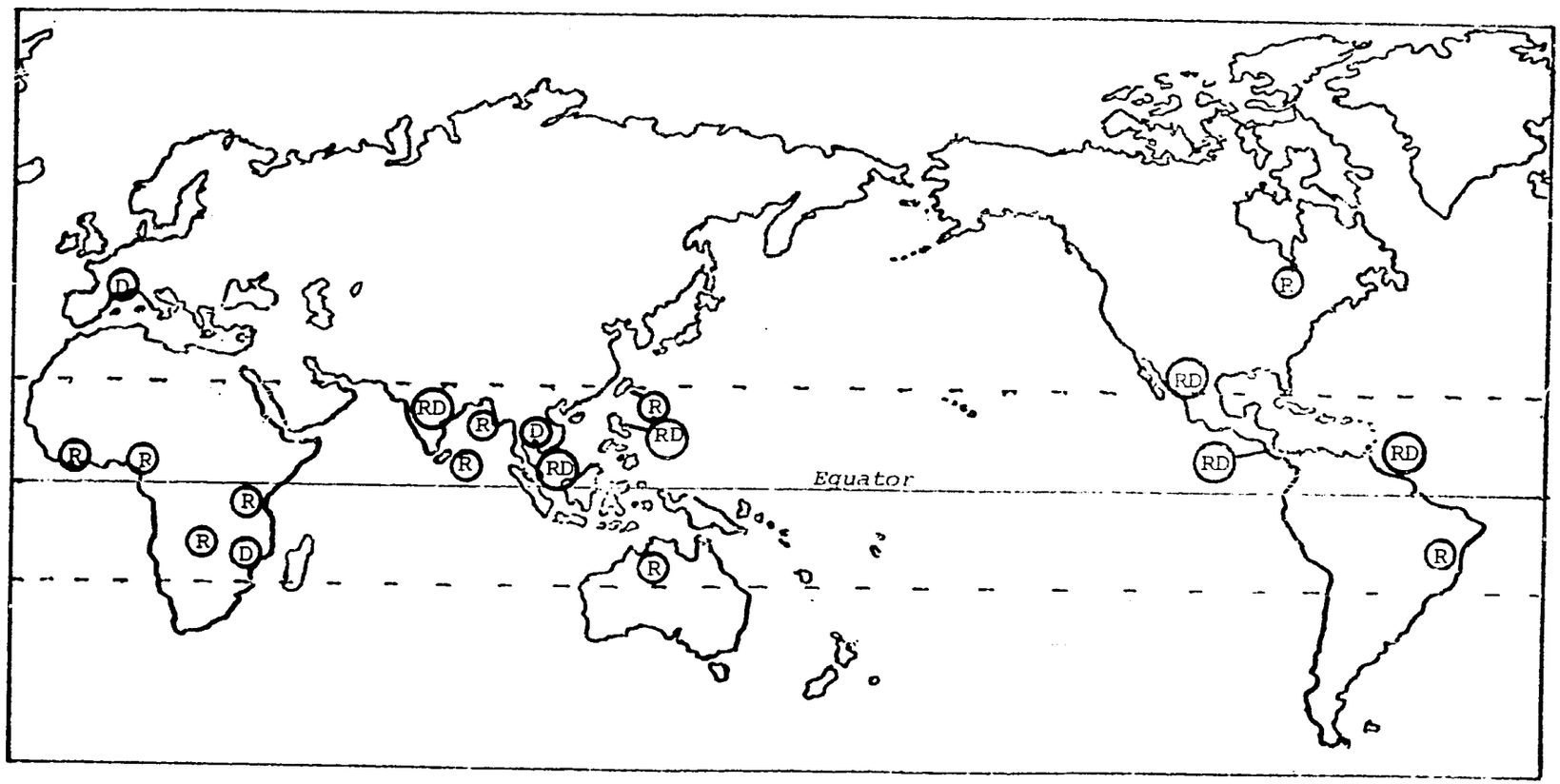
1) Strain Collection: (Reyes/Somasegaran/Kato)

The project acquired an additional 244 strains during the third year, mostly proven strains from cooperating laboratories[1].

The working collection now includes strains isolated from all of the primary target legumes (except *Vigna mungo*: inactive collection only), most of the secondary target legumes, plus many other non-target legumes (Table 1). Thus the collection now has a very broad germplasm base which makes it possible to easily assemble a wide range of *Rhizobium* strains for screening on nearly all of the legumes of interest.

As before, most of the strains are being maintained on dessicated ceramic beads for reliable storage and ease of access.

[1]Countries which contributed strains included France (2), India (30), Malaysia (101), Mexico (6), Panama (2), Philippines (8), Rhodesia (9), Thailand (3), and West Indies (12). The remainder were isolated by NifTAL.



Ⓡ Recipients from, and Ⓓ Donors to, the NifTAL culture collection of *Rhizobium* strains.

Australia	India	Nigeria	Taiwan
Bangladesh	Kenya	Panama	Thailand
Brazil	Liberia	Philippines	Trinidad
Canada	Malaysia	Rhodesia	Turkey
France	Mexico	Sri Lanka	Zambia

Table 1. Cultures maintained in the NifTAL *Rhizobium* collection, classified on the basis of original legume host.

Target Grain Legumes

Arachis hypogaea	38
Cajanus cajan	30
Cicer arietinum	49
Dolichos lablab	17
Glycine max	134
Lens esculens	34
Phaseolus vulgaris	32
Vigna mungo	--*
Vigna radiata	59
Vigna unguiculata	43

Target Pasture Legumes

Centrosema spp.	27
Desmodium sp.	43
Glycine wightii	3
Leucaena leucocephala	11
Macroptilium atropurpureum	2
Stylosanthes spp.	23

Target Green-manure and Cover-crop Legumes

Canavalia sp.	3
Crotolaria sp.	12
Glyricidia sp.	--
Sesbania sp.	2
Stizolobium sp.	6

Target Minor Grain and Vegetable Legumes

Phaseolus lunatus	5
Voandzeia subterrania	3
Psophocarpus tetragonolobus	12
Vicia faba	8

*Recommended strains not from original host.

Other Legumes

Acacia spp.	7
Aeschynomene sp.	4
Albizzia moluccana	2
Alysicarpus viginalis	5
Anthyllis vulneraria	3
Arachis spp.	3
Argysolobium calycinum	1
Astralagus spp.	5
Bauhinia sp.	1
Calopogonium spp.	8
Caragana arborescens	7
Cassia sp.	16
Clitoria spp.	11
Colutea arborescens	2
Coronilla spp.	4
Desmanthus virgatus	1
Dolichos spp.	6
Erythrina indica	1
Galactia spp.	8
Galega officinalis	2
Indigofera spp.	11
Laburnum vulgare	1
Lathyrus maritimus	2
Lespedeza spp.	4
Lotononis bainesii	4
Lotus spp.	14
Lupinus spp.	21
Medicago spp.	29
Melilotus spp.	5
Mimosa spp.	4
Ornithopus sativa	1
Pachyrrhizus spp.	2
Phaseolus spp.	23
Pisum sativum	22
Pithocellobium jiringa	2
Psophocarpus palustris	1
Pueraria spp.	6
Robina pseudoacacia	1
Samanea saman	2
Serradilla spp.	3
Sphenostylis stenocarpa	5
Tephrosis spp.	2
Teramnus uncinatus	1
Trifolium spp.	51
Vicia spp.	10
Vigna spp.	16

2) Documentation: (Reyes/Somasegaran/Kato)

The strain-host index file and the permanent file, both developed by Dr. Reyes, have been updated to include approximately 1,000 strains of *Rhizobium*.

Mr. James Kato assumed the responsibilities of Assistant Curator of the Collection in midyear and has further refined the procedures for entering strains into the collection. About 95% of the cultures received can be authenticated on siratro (*Macroptilium atropurpureum*). The others are authenticated on the original host (e.g. *Cicer arietinum* (7), *Glycine max* (18), *Leucaena leucocephala* (23), *Macrotyloma axillare* (2), *Phaseolus vulgaris* (10), and *Vigna mungo* (20). The cultures are then also characterized on different media and stored on dessicated ceramic beads. A total of 282 cultures were thus authenticated and preserved on beads during the third year.

In addition, full documentation is prepared for those strains which have been screened for effectiveness (see following section) and are recommended for further testing, and the information is sent to the World Data Center for Microorganisms for inclusion in their computerized data retrieval system.

3) *Rhizobium* Catalogue: (Reyes/Somasegaran/Munns)

The NifTAL Project staff compiled and published an abbreviated *Rhizobium* catalogue as an adjunct to a more comprehensive catalogue published under the auspices of the World Data Center for Microorganisms (WDCM).

Although arrangements were initiated for the publication of a comprehensive *Rhizobium* catalogue under contract with the WDCM, publication of this proposed catalogue has been delayed indefinitely due to slow responses from curators of other collections as well as staffing problems at the WDCM.

Thus a catalogue of selected tropical *Rhizobium* strains in the NifTAL collection was completed and distributed in December, 1977/January, 1978. The catalogue was revised somewhat in April, 1978 (Appendix I), but this was not widely distributed since a major revision is scheduled for September, 1978, incorporating the results of screening trials in progress during the summer.

The catalogue lists only those strains which have proven to be effective on target legumes and which the NifTAL staff recommends for further testing in the field and/or with additional legume varieties. Strains listed in the catalogue are all fully documented at the WDCM, and it is anticipated that duplicate cultures will be provided to the USDA Beltsville collection once they are prepared to receive them.

4) Dissemination of Strains: (Reyes/Somasegaran/Kato)

Please refer to section on Dissemination and Utilization.

PURPOSE Ib. Screening for Superior *Rhizobium* Strains.

A. Working Objectives

1) To select one or more superior strains of *Rhizobium* for 3 or 4 target grain legumes, each to have one or more of the following characteristics:

a) Highly effective on one or more target legumes.
b) Infective and persistent in acid soils.
c) Infective on host legume in competition with native rhiozbia.
d) Tolerant to high soil temperature.
e) Effectively nodulate a wide spectrum of legume hosts.
f) Have growth characteristics which are compatible with inoculant manufacture.

2) To determine other strain characteristics such as growth rate on YMA, reaction, etc. for all strains of interest (500 strains minimum).

3) To distribute the information on strain performance and characteristics to workers in the tropics by means of a catalogue published in cooperation with the World Data Center for Microorganisms.

B. Progress to Date

1) Screening for Superior Strains

a) Effectiveness on Target Legumes: (Reyes/Somasegaran)

In addition to the 136 strains tested in the previous year, a total of 284 strains have been screened for the target legumes listed in Table 2.

This testing program has provided the basis for a revised catalogue of *Rhizobium* strains scheduled to be issued in September, 1978. Most of the strains thus selected in the greenhouse (or growthroom) require additional field testing and, in some cases, tests using different cultivars of the same species before they can be recommended for commercial use. However, it is noteworthy that two broad spectrum cowpea-type strains (CB 756 and Nitragin 176A22) perform consistently well in many trials involving legumes commonly nodulated by cowpea-type rhizobia and that these strains are already in commercial

Table 2. Greenhouse selection of effective *Rhizobium* strains for tropical legumes.[1/]

Target Legume	Investigator	No. Strains Tested	No. Effective Strains
Major Grain Legumes			
1. *Arachis hypogaea*	Munns/Singleton	16/12	4/4
2. *Cajanus cajan*	Reyes	14	3
3. *Cicer arietinum*	Reyes	53	6
4. *Glycine max*	Munns	14	14
5. *Lablab purpureus*	Somasegaran	23	4
6. *Lens culinaris*	May	30	5
7. *Phaseolus vulgaris*	Reyes	27	12
8. *Vigna radiata*	Munns	41	11
9. *Vigna mungo*	Reyes	37	9
10. *Vigna unguiculata*	Munns	19	5
Minor Grain Legumes			
11. *Dolichos biflorus*	Somasegaran	22	4
12. *Phaseolus acutifolius*	Somasegaran	20	3
13. *Phaseolus angularis*	Somasegaran	22	5
14. *Phaseolus lunatus*	Somasegaran	21	1
15. *Psophocarpus tetragonolobus*	Somasegaran	6	6
16. *Voandzeia subterranea*	Somasegaran	21	6
Forage Legumes			
17. *Leucaena leucocephala*	Reyes	24	4

1/ Leonard jar assays (except *Cicer* and tests by Munns) using washed sand or vermiculite growth medium and a N-free nutrient solution. Results compared with an uninoculated control and (in most cases) a plus N control (≥70 ppm N). Three or four replications. Two or more plants per assembly. Some *Cicer* strains tested in pots containing washed sand in the growth room with variable replication. Tests by Munns were conducted in acid- and lime-amended Josephine and/or Goldridge soil.

Summary of Table 2 Findings

1. *Arachis hypogaea* (peanut): No acid-tolerant strains were found among 16 strains in a preliminary test. There were significant cultivar interactions among the top 3 strains.

2. *Cajanus cajan* (pigeon pea): Promiscuous, effectively nodulated by other cowpea-type rhizobia. All strains isolated from *C. cajan*, except one, were less effective than isolates from *Canavalia sp.* and *Desmodium uncinatum*.

3. *Cicer arietinum* (chickpea): Quite specific but there is wide variability in the effectiveness among homologous isolates. Only isolates from *C. arietinum* formed nodules. Few but large nodules were formed by the effective strains.

4. *Glycine max* (soybean): 13 out of 14 strains were both highly effective and highly tolerant of acid Josephine soil. USDA 110 was highly effective and only slightly less acid tolerant.

5. *Lablab purpureus* (hyacinth bean): Very promiscuous but different strains show a wide range of effectiveness. Some large red nodules were quite ineffective.

6. *Lens culinaris* (lentil): Highly specific as 10 out of 30 strains of *R. leguminosarum* were ineffective. Highly effective strains were from diverse origins. Tests with multiple-strain inoculum showed up to 25% double-infection in the nodules.

7. *Phaseolus vulgaris*: Very specific; nodulated only by isolates from *P. vulgaris*. But there is wide variation in the effectiveness of different isolates.

8. *Vigna radiata* (mung bean): 15 out of 40 strains were ineffective. Four highly effective strains (out of 11) were tolerant of acid soil conditions, including CB 756 and a strain from Thailand.

9. *Vigna mungo*: Very promiscuous. Effectively nodulated by isolates from *Vigna radiata*, *Mucuna deerigianum*, and *Vigna frutescens*. Also effectively nodulated by broad spectrum CB 756; Nitragin 176A22 was only moderately effective.

10. *Vigna unguiculata* (cowpea): Three cultivars tested were all quite promiscuous as expected, but there was a wide range of effectiveness. CB 756 and THA 306 were tolerant of acid Josephine soil, but isolates from Nigeria were best on acid Goldridge soil.

11. *Dolichos biflorus* (horsegram): Quite promiscuous but no strains exceeded 65% of plus-N control.

12. *Phaseolus acutifolius* (tepary bean): Moderately specific, as it was not effectively nodulated by the standard broad spectrum cowpea strains. Effective strains were from *Psophocarpus*, *P. lunatus*, *and Lablab*.

13. *Phaseolus angularis* (adzuki bean): Moderately specific as there was a large group of totally ineffective strains. However it was effectively nodulated by both standard broad spectrum cowpea strains (CB 756 and Nitragin 176A22) plus isolates from *P. angularis* and *Desmodium uniflorum*.

14. *Phaseolus lunatus* (lima bean): Very specific. Effectively nodulated only by an isolate from *P. lunatus*.

15. *Psophocarpus tetragonolobus* (winged bean): Very promiscuous. However both standard broad spectrum cowpea-type strains were outstanding.

16. *Voandzeia subterranea* (bambarra groundnut): Promising but with a wide range in strain effectiveness. Standard broad spectrum cowpea strain (Nitragin 176A22) was outstanding, but strains from several other species were also effective.

17. *Leucaena leucocephala*: Not highly specific but nodulated only by fast-growing "cowpea-type" strains. Isolates from *Prosopis chilensis* and *Leucaena leucocephala* both formed effective nodules.

use. Even these fail miserably on certain other legumes in the same category, indicating the necessity for careful screening of strains for each legume of interest.

b) Tolerance to Acid Soils: (Munns)

The work begun earlier at the University of California--Davis by Dr. D. N. Munns was continued under the subcontract program. Some of the results are described earlier in Section Ib, under *Arachis hypogaea, Glycine max, Vigna radiata,* and *V. unguiculata*. Strains have been identified which combine acid-soil tolerance with high effectiveness for all the species except *A. hypogaea*. Individual strain data are shown in Appendix II. Additional strains are being tested for *A. hypogaea*.

The tolerance of strains to acidity and/or high soluble Al were evaluated using a liquid medium containing glutamate, mannitol, vitamins, and salts. About one-half of the approximately 60 strains tested could grow at low pH (4.5 to 4.6) or high Al (50 μM) but less than one-third of the strains could grow in the presence of both high Al and low pH (Table 3).

Comparison of performance in these tests with plant tests indicate that failure to grown in the high Al, low pH medium is a good indicator of failure to establish effective nodulation in soil at pH 4.6. We therefore have the basis for a simple prescreening test. However, some

Table 3. Tolerance of *Rhizobium* strains to stress during growth in liquid medium.

Strains sensitive to acidity (pH 4.5 to 4.6)

No Growth: TAL 207, 310 (CB1024), 440 (m5), 441 (m6), 442 (m7)*, 463 (IQ68), IQ108, IQ921, 176A38, Allen 527

Slow Growth: TAL 98, 236, USDA n9*

Strains sensitive to 50 μM Al at pH 4.5

No Growth: TAL 98, 171, 200, 236, 295, 303, 305, 306, 422, 423, 437 (m2), 443 (m8), 176A38, USDA n10, n9, n22

Slow Growth: 127D5, USDA h7, 281, 420, 102, cc709

Strains tolerant of 50 μM Al at pH 4.5

TAL 11, 163, 169, 170, 172, 173, 174, 175, 176 189, 209, 236, 307, 309 (CB756), 421, 425, 438 (m3), 439 (m4), 32H1, Allen 542, USDA h4, n12, n16

*Note: strains 442 and n9 have steep pH-response curves; grow well at pH 4.7 or above, though not at pH 4.5.

rhizobia that succeed in growing at low pH and high Al nevertheless fail to form nodules (or establish an effective symbiosis) in acid soil (about 1 in 5 strains of those tested on cowpea). This error is to be expected from the known existence of acid-sensitive steps beyond the initial rhizosphere-colonization phase of the symbiosis.

A similar liquid media was used to determine tolerances to low phosphate. Only about 10% of the strains tested were sensitive to low (5 to 10 μM P) phosphate compared to growth at 100 to 1000 μM P.

With mung, a small portion of strains that are tolerant on one host variety proved sensitive on another variety, and vice versa. This implies that comparative testing of host tolerances should be done with more than one strain of *Rhizobium* and comparisons of rhizobia should use more than one host variety.

Results from two types of experiments have indicated that there is considerable within-strain variation with respect to acid tolerance. Sequential dilution studies indicate that some strains can grow in stress media only if inoculated at levels of 100 or 1000 cells per culture. Plating of such strains onto a newly developed agar medium in which acid and aluminum stress can be maintained with the use of aluminum hydroxide and citrate-glutamate buffering indicates also that most of the rhizobial cells are sensitive, but a small minority (e.g. one cell per hundred or per thousand) are tolerant. This work provides a basis for isolating single tolerant clones from strains of mediocre tolerance. Work is in progress to select and test clones derived from several such strains. If these clones maintain this tolerance to aluminum stress through subsequent generations, this technique will make it possible to select strains for acid soils much more easily than heretofore.

Other soil acidity factors such as low calcium and high manganese levels were also evaluated. Tests of about 20 strains indicated that none of the strains were inhibited by calcium deficiency (50 μM) or manganese toxicity (200 μM) Calcium was shown to have a negligible protective effect against aluminum in an experiment with 3 strains at four levels of each variable.

Tolerance of rhizobial growth to salinity was evaluated by growing rhizobia in liquid medium containing up to 2% NaCl. There were significant differences among strains of cowpea rhizobia, *Cicer* rhizobia, *R. japonicum*, and *R. meliloti*. A preliminary greenhouse trial with *Cicer arietinum* indicates symbiotic sensitivity to salt greater than that of either symbiotic partner when grown separately. This work is being checked and expanded.

c) Selection of Highly Competitive *Rhizobium* Strains: (May/Bohlool)

Thirty strains of *Rhizobium leguminosarum* were screened for effectiveness on lentils, *Lens esculenta*. Fluorescent antibodies were prepared against five effective strains. The three most effective strains were tested for competitive ability in sterile vermiculite using the fluorescent antibody technique to identify rhizobia in nodule samples. Results of this experiment (Table 4a) proved two of the strains to be highly competitive. These two strains, NZP 5400 and Hawaii 5-0 (a strain isolated from Hawaiian soil), were further tested in a field competition experiment (Ustic Humitropept, pH 6.1) and were found to be equally competitive against each other and were able to overwhelm the ineffective indigenous population of lentil rhizobia (Table 4b).

A poster session was presented at the annual meeting of The American Society for Microbiology in May, 1978: S. N. May and B. B. Lohlool, "Application of Immunofluorescence to the Selection of Highly Competitive Strains of Lentil *Rhizobium*" (Abstract, Appendix IV). A write-up on a portion of this work entitled "*Rhizobium* Ecology" also appeared in the March, 1978, CST Newsletter.

Studies on the persistence of these two strains in Indonesian, Philippine, and African soils is presently underway.

d) Tolerance to High Temperature: (Somasegaran/Hoben)

Because *Rhizobium* inoculants in the tropics are frequently exposed to high temperature during shipping, merchandizing, and/or final use, the tolerance of potential inoculant strains to high temperature is an important consideration.

Ten slow-growing (cowpea-type) strains which had been previously screened for effectiveness were tested in a peat-carrier for 14 weeks at two temperatures: 28°C (moderate tropical ambient) and 37°C (high tropical ambient). At 28°C all the strains maintained relatively high numbers (10^9 to 10^{10}) throughout the 14-week period. At 37°C most strains increased in number ($>10^{10}$) and then declined slowly to about 10^7 to 10^8. However, three of the 10 strains were very temperature sensitive and declined to nearly zero within 2 to 6 weeks. At least one of these strains (TAL 655) was isolated from a cool, high elevation site in Malaysia.

Table 4a. Percentage of lentil nodules formed by inoculated strains of *Rhizobium leguminosarum* in sterile media.

Inoculum Strains*	Strains Recovered in Nodules (Percent of Total)						
	A	B	C	A&B	A&C	B&C	A&B&C
Uninoculated control	-	-	-	-	-	-	-
NZP 5400 (A)	100	-	-	-	-	-	-
Hawaii 5-0 (B)	-	100	-	-	-	-	-
Nitragin 128A12 (C)	-	-	100	-	-	-	-
Mixture (A&B)	45	40	-	15	-	-	-
Mixture (A&C)	90	-	10	-	0	-	-
Mixture (B&C)	-	65	10	-	-	25	-
Mixture (A&B&C)	61	26	0	0	0	13	0

*10^6 bacteria (single or mixed) were added to each lentil seedling.

Table 4b. Percentage of lentil nodules formed by introduced strains of *Rhizobium leguminosarum* in the field.

Inoculum Strains	Strains Recovered in Nodules		
	NZP 5400	Hawaii 5-0	Both Strains
Unincoulated control	12	6	--
NZP 5400	100	0	--
Hawaii 5-0	0	100	--
NZP 5400 + Hawaii 5-0	32	30	38

This experiment has shown the importance of such a temperature-screening prior to release of strains intended for use in the tropics. Further work will therefore be carried out in 1978-79, using more strains and a greater range of temperatures. A possible beneficial effect from high curing temperature for the tolerant strains will also be explored further.

d) Potential for Selection within Strains: (Hoben)

The hypothesis that re-isolates from nodules after passage through an appropriate host outperform old stock cultures was tested using large Leonard jars in the greenhouse. Passage of *B. japonicum* (soybean rhizobia) through up to three series of plant nodules gave significant improvement in the performance of strain USDA136b (=TAL 379 and CB1809) (See Appendix IIb). There was no benefit for substituting a non-homologous host (cowpea) in this case.

There was no benefit for one or two passages of cowpea rhizobia through cowpea, but there was a significant improvement in strains which were subjected to three cowpea passages or through one passage through common bean plus one passage through cowpea. Passage of *R. phaseoli* was beneficial only when the strain was passed through and re-isolated from both cowpea and bean nodules.

These results generally support the hypothesis. In no case did passage decrease effectiveness. However, some strains responded significantly to plant-nodule passage whereas others did not. It is not known whether the passage merely restores responsive strains to their original effectiveness, or whether it selects the most effective organisms from a variable population and thereby actually improves strain effectiveness over the original isolate.

In either case, this technique provides a very useful tool to ensure that the best possible variant of a strain is carried forward, even with non-responsive strains (since effectiveness does not decline).

e) Field-testing of *Rhizobium* Strains: (Somasegaran/Guevarra)

In addition to the strain trials with soybean previously reported (Paia, Maui: no differences among a group of effective strains), a second test of eleven soybean *Rhizobium* strains was conducted at the Iole site of the Benchmark Soils Project at Kohala on the island of Hawaii.

Total yield of plant tops at the early podding stage was used as the measure of strain performance. Two strains

significantly exceeded the control: USDA 138 (TAL 377) and USDA 136 = CB1809 (TAL 379), but there were no significant differences between these strains and the others in the group (Table 5). A nitrogen-fertilized treatment (150 kg/ha split-application) was significantly better than the symbiotic treatments, indicating that the symbiotic plants were unable to fix sufficient N for maximum yield under the cool, damp conditions prevailing during the trial.

A trial to compare a number of cowpea strains was subsequently planted at the same site, but had to be abandoned when the irrigation source dried up during drought conditions.

A *Rhizobium* strain trial for lentil bacteria was initiated in conjunction with the fourth training course. Seven strains of *R. leguminosarum* selected by Ms. Sheila May (see Section Ib, Table 1), were tested at the Paia site late in the cool season. The plants did not flower at this site and vegetative yield at 15 weeks was therefore used as the measure of strain performance. Three strains were significantly superior at both an early sampling period (4.5 weeks) and at 15 weeks: TAL 640 (UH:I-11), TAL 635 (UH-B8[illegible]), and TAL 639 (UH:I-7). All of these strains were isolated on Oahu, Hawaii. Although early comparisons were restricted by a moderately high level of nitrogen in the soil at planting time, the plants inoculated with the best strains performed better than both plus- and minus-N controls at 15 weeks, with an average weight of 2.25 kg/plant for the top three strains.

A fourth field trial is described in Section IIa on inoculation techniques.

2) Laboratory Characterization of Strains: (Kato)

The major emphasis has been on methodically rechecking and authenticating the NifTAL *Rhizobium* collection which now numbers over 1,000 strains and isolates. This work is well along with an anticipated completion date of March, 1979.

PURPOSE IIa. Inoculation Techniques.

A. Working Objectives

1) To maximize the nodulation of legumes by an introduced *Rhizobium* through increased efficiency of inoculation and through the identification of more competitive strains of *Rhizobium*.

Table 5. Dry weight and nitrogen yields of soybean cv. Jupiter grown in association with different *Rhizobium* strains at Kohala, Hawaii. Cool season 1978 crop. Average of four replications. Harvested at early podding stage.

	Treatment		DW of Tops	Percent	N Yield of Tops
	TAL No.	Source	g/Plant	Nitrogen	mg/Plant
	N-150	---	16.3 a	3.73 cde	619 a
8	377	USDA 138	9.9 b	4.23 a	418 b
3	379	USDA 136	8.9 bc	4.45 a	393 b
4	211	Thailand	9.4 bc	4.00 bc	366 bc
5	103	61A118	8.3 bc	4.13 ab	342 bc
7	102	USDA 110	7.8 bc	4.31 ab	377 bc
10	---	Allen 527	8.7 bc	3.97 bc	316 bc
11	183	61A76	7.8 bc	4.06 bc	314 bc
12	---	Brazil 587	9.0 bc	3.55 ef	308 bc
13	216	Thailand	7.8 bc	3.95 bcd	306 bc
9	411	THA-3	8.5 bc	3.60 def	305 bc
6	206	Thailand	8.7 bc	3.31 fg	287 bc
	N-0	---	6.6 c	3.09 g	239 c

NOTE: Data in each column followed by the same letter are not different at approximately the 5% level of significance using the Bayes LSD test.

2) To develop methods that will increase the density and improve the survival of the applied *Rhizobium* in the vicinity of the seed.

B. Progress to Date

1) Inoculation Methodology: (Reyes)

A cowpea field experiment was laid out to determine the recovery (in the nodules) of an applied *Rhizobium* inoculum into a soil containing a high background population of native rhizobia. Numbers of rhizobia in the soil capable of nodulating *Macroptylium atropurpureus* (siratro) were estimated using the plant infection technique. The native population of *Rhizobium* was rather high (10^3/g at the surface and 10^4/g below the surface) but this is probably typical of many tropical situations where legumes have been grown for many years.

Three strains of rhizobia were compared at approximately the same level of inoculum coated onto the seed using a standard method. One of the three strains was also applied using different inoculum concentrations (10^3 to 10^9 per seed) and placements (various combinations of seed coating, band placement, and mixing with the soil). Table 6 shows the various treatment combinations and their effects on nodulation.

There were no differences in yield (top weight or grain) since the native rhizobia were highly effective. However, there were significant treatment differences with respect to the number of nodules found by the introduced strain (Table 6). Using the standard seed coating method (10^3 to 10^5 cells/seed) only a few nodules were formed by all except CIAT 239, but none significantly exceeded the control. As the numbers were increased (Fig. 1), recovery also increased to a maximum of about 34%. Few nodules were formed by the introduced strains until inoculum levels significantly exceeded the population of native rhizobia below the soil surface. In addition, there was strong evidence that placement of the inoculum near the seed was more beneficial than mixing with the soil.

This experiment has given valuable preliminary information regarding both the quantities and techniques required to achieve high rates of nodulation by introduced strains. Additional work will be required to reliably achieve maximum recovery with a minimum of inoculum.

Table 6. Nodules formed by introduced rhizobia as affected by *Rhizobium* strain and inoculation treatment.

Treatments		Rhizobia Applied		
Strain	How Applied	To Seeds	To Soil[a/]	% Nodule Recovery[d/]
Control	----	---	---	---
CIAT 239	Peat Pellet[b/]	2×10^5	---	0 a
176A22	"	1×10^5	---	2.9 a
CB 756	"	1×10^3	---	3.8 a
"	"	5×10^4	---	5.8 ab
"	Peat + Sticker Coated[c/]	3×10^5	---	9.6 bc
"	Peat Pellet	4×10^5	---	16.3 cd
"	Soil Band	---	1×10^7	16.3 cd
"	Band + Pellet	4×10^5	1×10^9	33.7 e
"	Soil Mix	---	1×10^7	11.5 bcd
"	Mix + Pellet	4×10^5	1×10^9	28.8 e

a/ Per 2.5 cm linear length of row (equivalent to one seed).

b/ Coated with gum arabic sticker followed by peat-base inoculum.

c/ Coated with gum arabic-peat inoculum mixture.

d/ Data followed by the same letter are not significantly different at approximately the 5% level of significance using the Bayes LSD test.

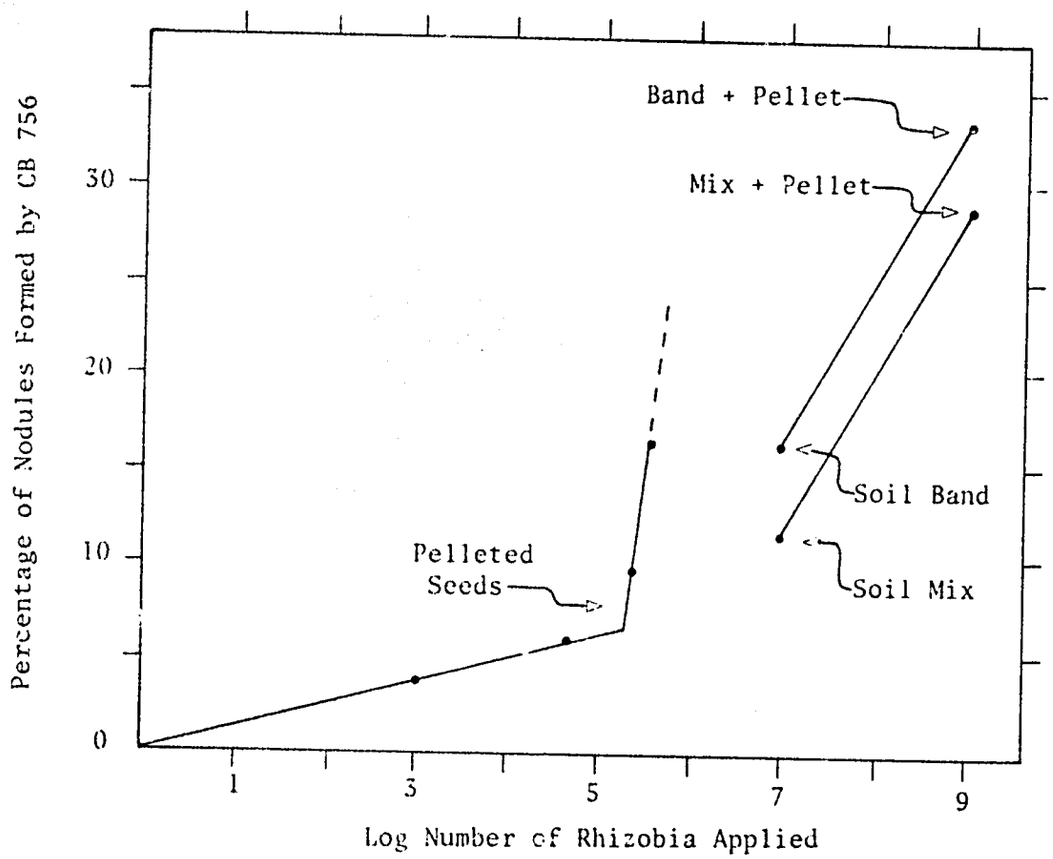


Figure 1. Recovery of rhizobia in cowpea nodules as influenced by inoculum rate and method of application.

PURPOSE IIb. Cultural Systems for Maximizing Symbiotic Nitrogen Fixation.

A. Working Objectives

1) To better define the role of legumes and their residues as N sources in cropping systems, and to devise and test methods designed to maximize the N contribution of legumes under conditions of minimum inputs.

2) To determine the effects of soil acidity (especially high soil aluminum and low soil calcium), phosphorous nutrition, "starter" nitrogen, and competition for sunlight and nutrients by associated crops on the nitrogen contribution by legumes.

B. Progress to Date

1) Legumes in Cropping Systems: (Guevarra/Kitamura/Whitney)

A field experiment was continued at Paia for a second year with modified treatments. Since legume yields were quite low in the previous experiment where standard maize spacing was employed, two approaches were used to achieve a better balance of maize and legumes: 1) three maize spacing/population treatments, all with 1 meter row spacing: a) 25 cm between hills, 1 plant/hill; b) 50 cm between hills, 2 plants/hill; and c) 50 cm between hills, 1 plant/hill; and 2) two maize types: H68 sweet corn, and H610 field corn. Two legumes were employed: Clark 63 soybean and California Blackeye #5 cowpea, and the results were compared with maize which received no nitrogen or 100 kg N/ha as urea.

The results of the field experiment (Table 7, and Fig. 2) indicate that the best balance would be achieved by growing the legume and the maize crop in separate plots. If the plots were alternated, the maize would then be able to utilize the nitrogen released from decaying legume residues. This is reinforced by the results of the previous year's experiment where wheat yields following pure stands of TVu1190 cowpea were nearly double (8.4 T/ha) the yields of wheat following maize (4.3 T/ha), and wheat following soybean in pure stand yielded 6.1 T/ha, 40% more than wheat after maize.

Nitrogen fixation by legumes grown alone and intercropped with maize was estimated by acetylene-reduction-assay at several growth stages. Nitrogen fixation was closely correlated with the yield of plant tops, and was severely inhibited by high maize population at the late-flowering stage (Table 7). Soybean N-fixation levels at late flowering were the same in the low-maize population treatment (c) as in pure stand, but this may have been due to delayed maturity of the

Table 7. Yields of maize, cowpea, and soybean grown singly and as maize-legume intercrops. Summer 1977 crop. Average of four replications.

Treatment		Tons DM per ha.	Days to Harvest	Maize Ear Length (Legume Pod No.)
Maize Alone		4.35 b	94	35
Maize + N_{100}		5.80 a	94	42
Soybean Alone		3.70 b	88	(28)
Cowpea Alone		2.76 c	74	--
Maize/Soy:	Maize	3.09 (c)	94	23
	Soybean	1.06	88	(22)
	Total	4.15		
Maize/C'pea:	Maize	3.02 (c)	94	22
	Cowpea	0.98	74	--
	Total	4.00 b		

Maize: 1 m x 25 cm alone; 1 m x 50 cm mixtures; cv. H610

Soybean: 50 cm x 2.5 cm alone; 1 m x 2.5 cm mixtures; cv. Clark

Cowpea: 50 cm x 5 cm alone; 1 m x 5 cm mixtures; cv. Blackeye 5.

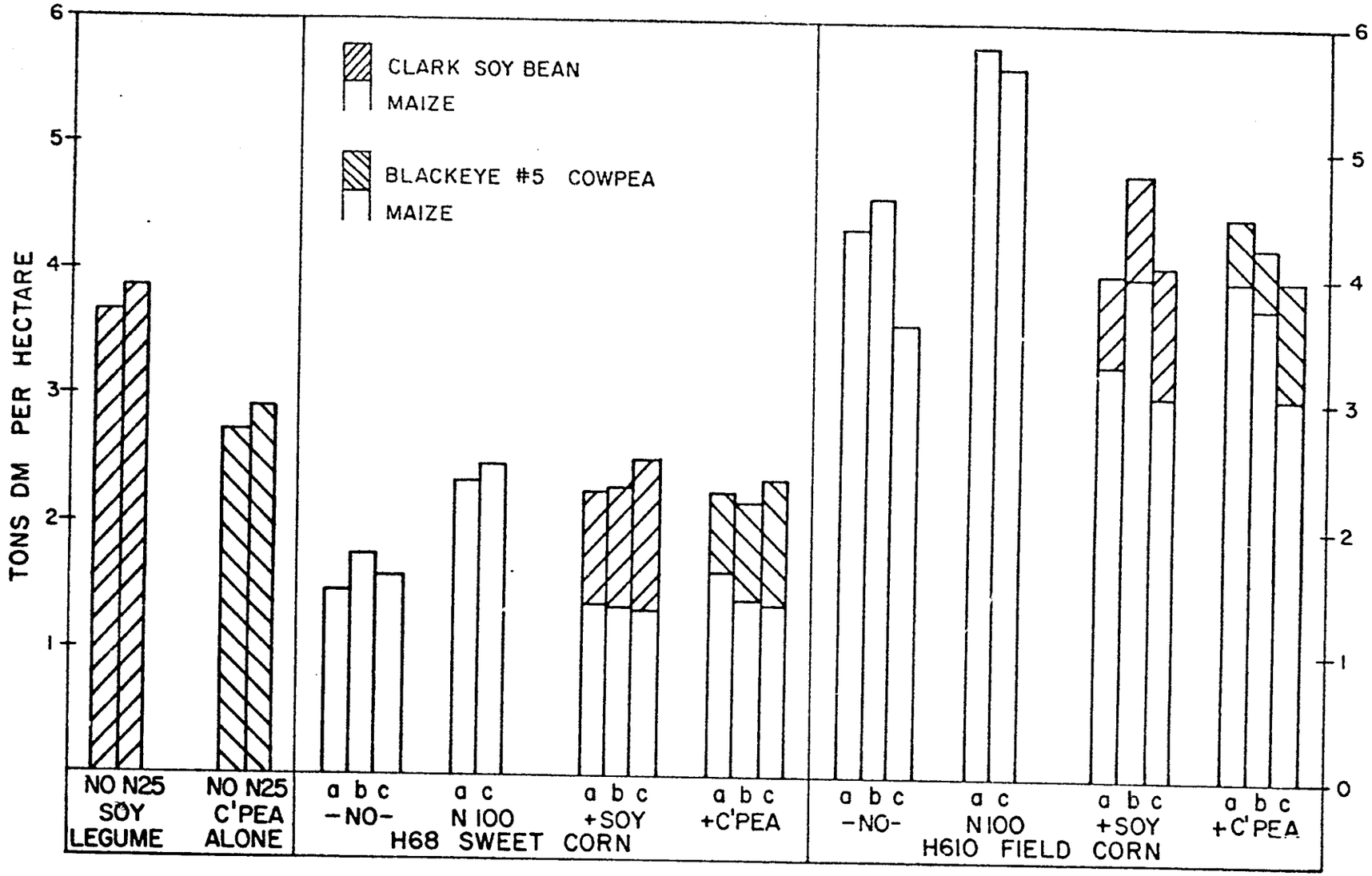


Fig. 2. Yields of maize, soybean, and cowpea grown singly and in maize-legume mixture employing different maize spacings.

intercropped soybean. However, cowpea was lower in N-fixation in all intercropping treatments than in pure stand. Cowpea continued fixing N at a relatively high rate late in its growth cycle, whereas soybean N-fixation at late flowering was only 50% of the rate observed at the early-flowering stage. There was some evidence of increased N-fixation due to a starter application of 25 kg N/ha in both legumes, but the effect persisted to late flowering only in cowpea (Table 8).

Several pot experiments were conducted in the greenhouse to follow up on phenomena observed in the field. The depression in maize yield due to intercropping with stylo (*Stylosanthes guianensis* cv. Endeavor) which was observed in the field was investigated further by growing stylo in 18 liter pots singly and together with maize (and later with *Setaria sphacelata* cv. Narok). Plus- and minus-N and plus- and minus-P treatments were also included. In contrast to the field experience, maize severely suppressed the stylo in all treatments (Table 9). There was a tendency toward enhanced maize yield in the presence of stylo. There were significant responses to both N and P in all treatments. However, when setaria was subsequently planted, both setaria and stylo responded to applied N but not to P (Table 10). There was a strong inverse relationship between setaria yield and stylo yield (Fig. 3), indicating a probable alleleopathic effect of setaria upon stylo growth. We hope to pursue this further to learn whether this effect is primarily exerted upon the host or the rhizobial component of the symbiotic association.

In another pot trial, leaf material from a number of legumes was added to the soil at two rates, either mixed with the soil or added as a "cube". Although it was anticipated that *Desmodium intortum* leaf would act as a slow-release N source due to its high phenolic content, there were no differences among the legumes (*D. intortum*, *Leucaena leucocephala*, stylo, *Cajanus cajan*, *Centrosema pubescens*, and *Macroptilium atropurpureum*) with respect to the nitrogen supplied to four successive harvests of setaria grass. Compared to urea, the amount of N harvested in the grass was 83% at the 200 kg N/ha rate and 68% at the 400 kg N/ha rate. The legume leaf material was most efficient at the lower level, both on an absolute basis and in comparision with urea.

In a third pot experiment, grass-legume combinations were grown in separate 18-liter pots in gravel-plus-vermiculite culture, and N-free nutrient solution was circulated alternately between each grass-pot/legume-pot combination. A grass-grass combination was included as a control. After planting, the grass (setaria cv. Narok) was harvested three times over a 95-day period, and the legumes were cut as needed at the same harvest dates. Five legumes were compared for their contribution of N (or other growth factors) to the associated setaria: Clark 63 soybean, California Blackeye cowpea,

Table 8. Dry matter and nitrogen yields and acetylene reduction activity of soybean grown alone and in combination with different maize spacing treatments. Summer 1977 crop. Average of four replications.

Treatments		DM/Plant (g)		Total N (mg)	C_2H_2 Red'n. μm/hr.	
		Tops	Nodules		Plant	g. Nod.
SOYBEAN						
Alone	N_0	25.1	.38	1020	16	43
	N_{25}	23.3	.37	910	16	43
w/Maize	a*	10.8	.16	420	6	37
	b	11.6	.19	450	6	32
	c	18.5	.34	750	14	43
COWPEA						
Alone	N_0	30.4	.31	850	36	111
	N_{25}	40.4	.27	1120	21	78
w/Maize	a*	17.4	.06	460	3	48
	b	25.5	.10	650	4	53
	c	27.7	.18	720	14	66

*Maize Spacing: a = 1 m x 25 cm (1 plant/hill)
b = 1 m x 50 cm (2 plants/hill)
c = 1 m x 50 cm (1 plant/hill)

Note: Soybean C_2H_2 activity is ≈ 50% of earlier levels.

Cowpea C_2H_2 activity is ≈equal to earlier levels.

Table 9. Greenhouse responses of maize and stylo to N and P when grown alone and in association.

Treatment	No N		Plus N	
	No P	Plus P	No P	Plus P
	g DW/pot			
Maize Alone	37	73	58	188
Stylo Alone	2.8	5.6	3.7	10.0
Mixture:				
Maize	41	80	67	184
Stylo	0.6	0.3	1.1	0.5
Total	42	80	68	185

Table 10. Greenhouse responses of *Setaria ancepts* and stylo when grown alone and in association.

Treatments	Setaria/Stylo Regrowth		Setaria Regr./Stylo Regr.	
	No N	Plus N	No N	Plus N
Setaria Alone	0.9	21.9	1.3	6.2
Stylo Alone	10.8	31.1	3.0	8.3
Mixture:				
Setaria	0.6	14.3	0.8	4.4
Stylo	0.9	4.9	0.5	1.0
Total	1.5	19.2	1.3	5.4

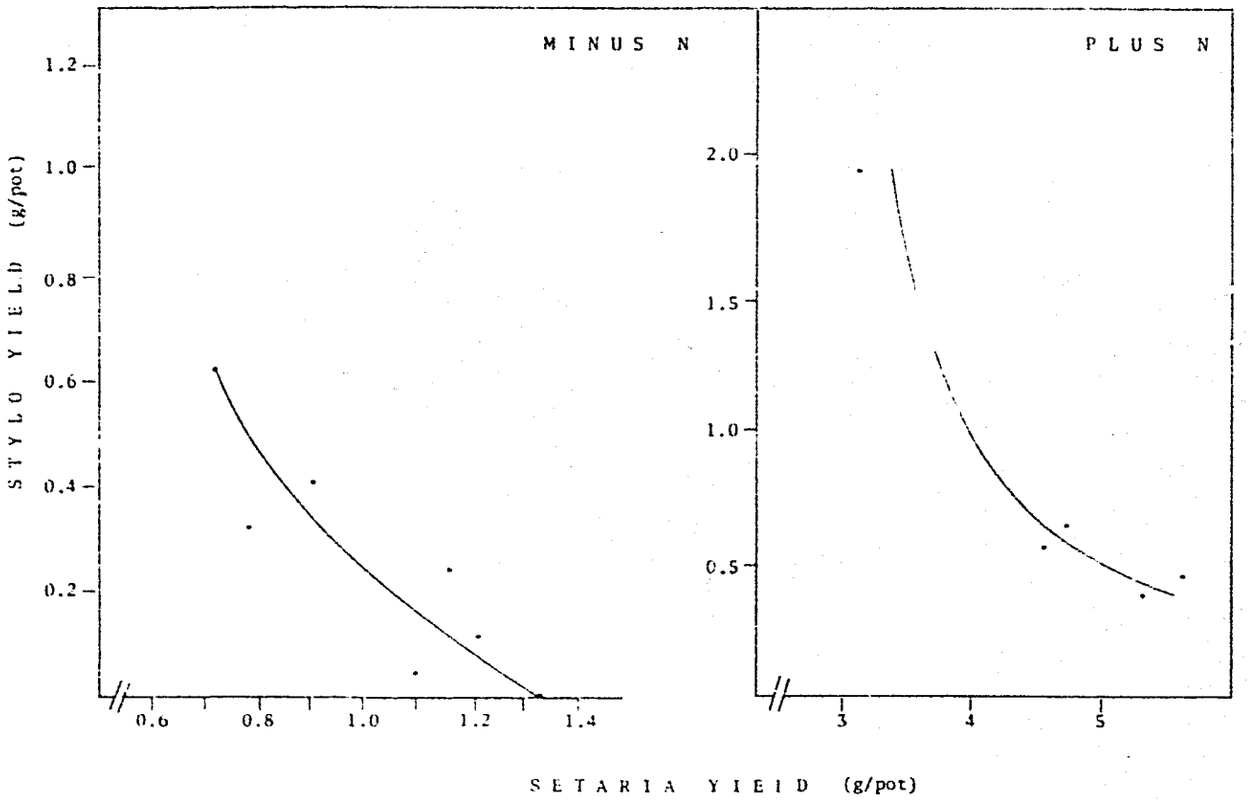


Fig. 3. Yield of Endeavor stylo in relation to yield of Narok setaria grown in the same 18ℓ pot.

Greenleaf *Desmodium intortum*, Hawaiian Giant *Leucaena leucocephala*, and Endeavor stylo. The results (Fig. 4) showed that both soybean and cowpea were very strong competitors for N during growth. However, after harvest, large amounts of N were released to the grass, more so by soybean than by cowpea, presumably because cowpea began to regrow from the base of the plant.

Greenlead desmodium yields averaged nearly 350 mg/day of dry weight increase without competing for N with the setaria. In addition it improved setaria yield at the final harvest, probably due to nitrogen compounds released to the solution immediately after cutting. Leucaena averaged 80 mg N per pot per day, also without competing with the setaria. There was also a minor but consistent enhancement of setaria yield at all harvests.

Stylo, on the other hand, was severely stunted in association with setaria, averaging only 10 mg/day. Thus the total for the two pots (stylo + setaria) was approximately equivalent to the total for the setaria + setaria combination. Apparently there was an alleleopathic effect of setaria upon stylo which did not permit the stylo to make any contribution to the mixture.

This experiment provides firm evidence that rapidly growing grain legumes such as soybean and cowpea do not provide nitrogen to an associated crop, but rather compete strongly for any available soil nitrogen. However, after harvest, N is quickly released to a subsequent crop. On the other hand, forage legumes such as desmodium and leucaena do not compete for soil N, and may provide significant amounts of N to an associated grass after repeated defoliation.

These data reinforce the results of the field experiments which suggest that maximum benefit from grain legumes is achieved by rotation rather than by intercropping. Intercropping with forage legumes may be a very promising approach for exploiting legumes in polycrop systems if a market for the legumes exists or if they may be fed to livestock.

2) Lime and Fertility Effects on N-fixation: (Stockinger/Cassman)

An additional experiment was carried out on the lime gradient site used previously for determining the lime response to a wide range of tropical legumes. Up to four varieties of each of a fewer number of legumes were employed in order to evaluate intra-specific variability. Maize was also planted to assess nitrogen availability at the different pH levels.

The maize showed severe nitrogen deficiency symptoms at low pH (5.0 to 5.6) and no nitrogen deficiencies at high pH.

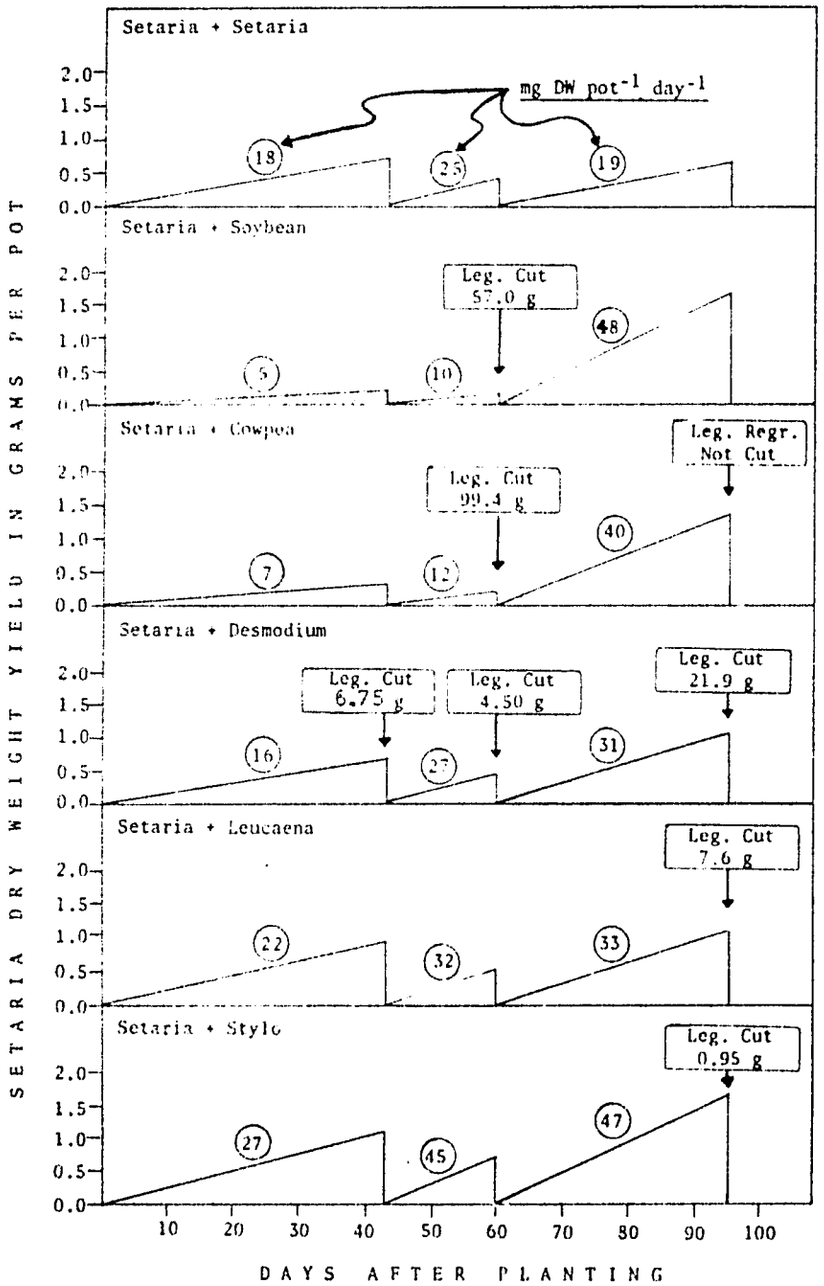


Fig. 4. Yields of Narok setaria perfused with solution from pots containing various legumes (and a setaria control).

This was obviously a nitrogen response and not a response to pH per se. The maize yield curve in Fig. 5 also shows a strong response to the increased availability of nitrogen with increasing pH.

The yields of mungbean, soybean, and peanuts reached their maximum yields at about pH 6.0. Yields were approximately 60 to 70% of the average maximum at pH 5.0. There were little or no differences in yield between Berken and a Philippine mungbean; between Jupiter, Bossier, Lee, and Clark soybeans; or between Starr, Florunner and Florigiant peanuts at pH 5. However, Philippine mungbean, Lee soybean, and Florgiant appeared to have higher yield potential above 6.0. The maize yields indicated that nitrogen release was confounded with pH. However, the legume data at the lower pH levels appears to be a reasonably valid description of the response since the N released from the soil was very small. Above pH 5.3, the response could be attributed to "starter N" effects since the N release was significant but was exhausted well before the legume harvest.

This experiment, together with soil analyses, has shown that a) liming a Humoxic tropohumult can release large quantities of nitrogen: b) that a very small increment of lime reduces the active aluminum to a low level; c) soybeans, mungbeans, and peanut are quite tolerant of acid soil low in aluminum.

The effect of phosphorous and nitrogen fertilizers on N fixation by soybean and cowpea was also studied in the greenhouse using pots perfused with nutrient solution containing various levels of nitrate and phosphate. At the highest P levels, the symbiotic (zero N) plants yielded only one-half as much as those grown with high N fertilizer. This was related to poorer root development in the symbiotic plants which were characterized by both reduced root mass and fewer fine lateral roots. This could have important implications for plant growth in the field (e.g. ability to scavenge for nutrients such as phosphorous and moisture), and an experiment has been planted to evaluate these effects in the field using both soybean and cowpea.

PURPOSE III. Training in *Rhizobium* Technology.

A. Working Objectives

1) To train LDC personnel in *Rhizobium* technology so that they may more effectively contribute to *Rhizobium* work in their own countries.

2) To build collaborative relationships in *Rhizobium* testing with the trainees and their employing institutions.

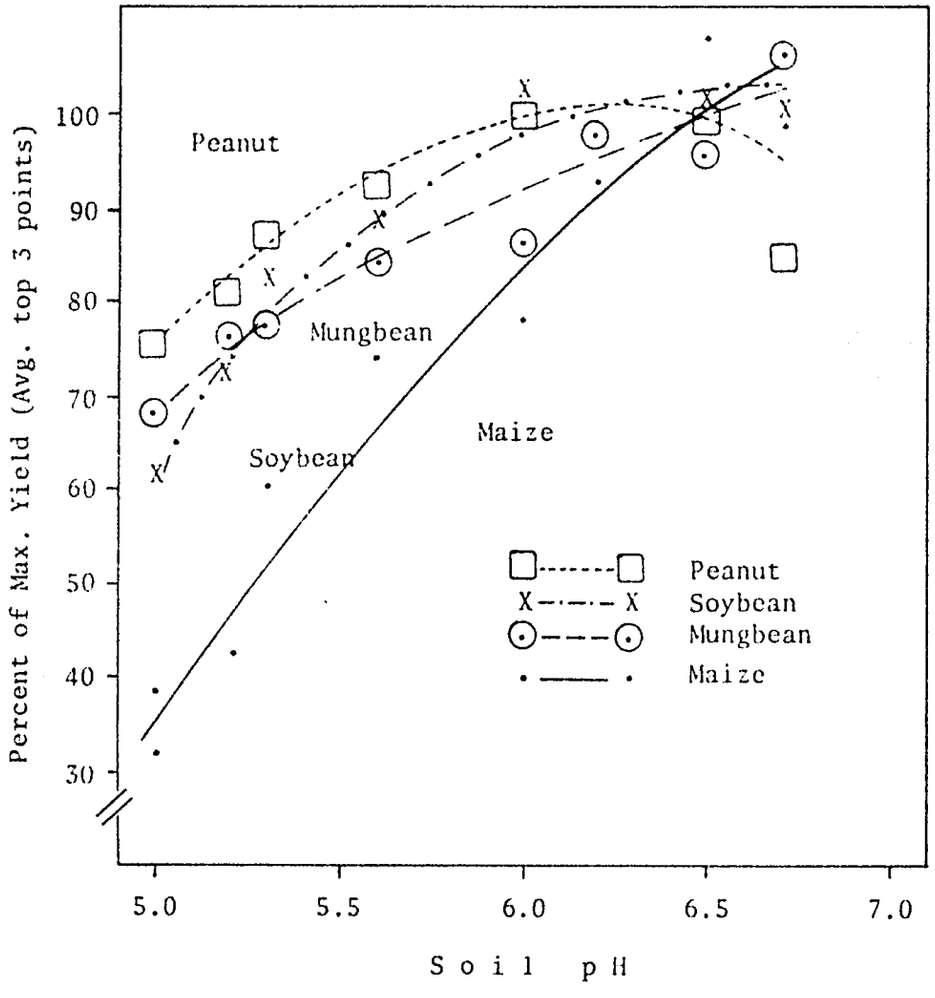


Figure 5. Relative yield of, maize, soybean, mungbean and peanuts with respect to soil pH.

3) To increase awareness by the NifTAL staff of problems and potentials of *Rhizobium* work in LDC's.

4) To utilize the research program and facilities of the NifTAL Project for the training of graduate students as specialists in *Rhizobium* research, and to further the goals of the project through their research contributions.

B. Progress to Date

1) Training Course in *Rhizobium* Technology: (Reyes/Somasegaran/Hoben)

Two additional training courses were conducted in 1977-78, following the two successful courses held earlier. The courses followed the same general format developed by NifTAL consultant Professor Emeritus J. M. Vincent, but were revised to permit a greater orientation toward field trials. The third course was conducted July 11 to August 19, 1977 by the NifTAL staff assisted by consultant R. W. Weaver of Texas A & M University, and the fourth course was conducted successfully February 6 to March 17 by the NifTAL staff. In all the courses, however, additional resource persons such as Manoa-based faculty and visiting scientists were invited to make presentations and their contributions have been very helpful.

A total of 25 trainees have now received intensive training in *Rhizobium* technology and most of these will be in positions to become excellent cooperators in the proposed network to field test *Rhizobium* strains under different tropical environments. Trainees were from the following countries: Philippines (3), Indonesia, Malaysia, AVRDC (Taiwan), Thailand (2), Bangladesh, India (2), Sri Lanka (2), Turkey, Egypt, Kenya, Tanzania (2), Nigeria (2), Ghana, Trinidad, Panama, Mexico, and USA (NifTAL graduate research assistant). (See map, p. 39)

Trainees during the third contract year were:

Training Course No. 3 Participants:
July 11 to August 19, 1977

1) Miss Angela Almendras
Department of Agronomy & Soils
Visayas State College of Agriculture
Baybay, Leyte, 7127
Philippines

2) Mr. Ronald Ferrera-Cerrato
Departamento de Microbiologia
Escuela National de Ciencias Biologicas
APDO 4-870
Mexico 4, D. F.

3) Miss Neeru Narula
Department of Microibology
Haryana Agricultural University
Hissar (Haryana), 125004
India

4) Mr. Lawrence O. Osa-Afiana (Student, Cornell Univ.)
Soil Science Section
Institute for Agricultural Research
Ahmadu Bello University
Samaru - Zaria
Nigeria

5) Mr. James Quashie-Sam (Student, Univ. of Guelph)
Crop Science Department
University of Ghana
Legon, Ghana
West Africa

6) Mr. Paul Singleton
Department of Agronomy & Soil Science
University of Hawaii
3190 Maile Way, Room 300
Honolulu, Hawaii 96822

Training Course No. 4 Participants:
February 6 to March 17, 1978

1) Mrs. S. K. D. Aloysius
Soybean Project
Regional Agricultural Research Station
Diyatalawa Road, Bandarwawela
Sri Lanka

2) Mr. Richard Graham
Department of Soil Science
Faculty of Agriculture
The University of the West Indies
St. Augustine, Trinidad

3) Dr. Velittin Gurgun
Ziraat Mikrobiyolojisi Kursusu
Ziraat Fakultesi
Ankara University
Diskapi/Ankara/Turkey

4) Ms. Blanca Hernandez
Universidad de Panama
Escuela de Biologia
Microbiologia
Panama, Republica de Panama

5) Dr. Md. Shamsul Hoque
Department of Soil Science
Bangladesh Agricultural University
Mymensingh, Bangladesh

6) Mr. W. A. Osinaike
Bacteriology Laboratory
P.M.B. 5042
Moor Plantation
Ibadan, Nigeria

7) Mr. Ernest Semu (Student, Univ. of Guelph)
Department of Soil Science and Agricultural Chemistry
University of Dar-es-Salaam
P. O. Box 643
Morogoro, Tanzania

2) Graduate Research Program: (Whitney/Sanford/Stockinger/others)

The four graduate research assistants employed by NifTAL were actively engaged in thesis research related to the project objectives during the third year and were learning new skills and concepts in the process. The research work being carried out by these students is as follows:

1) Mr. Kenneth Cassman: Effect of phosphorous nutrition on symbiosis (reported in Section IIb-B-2)

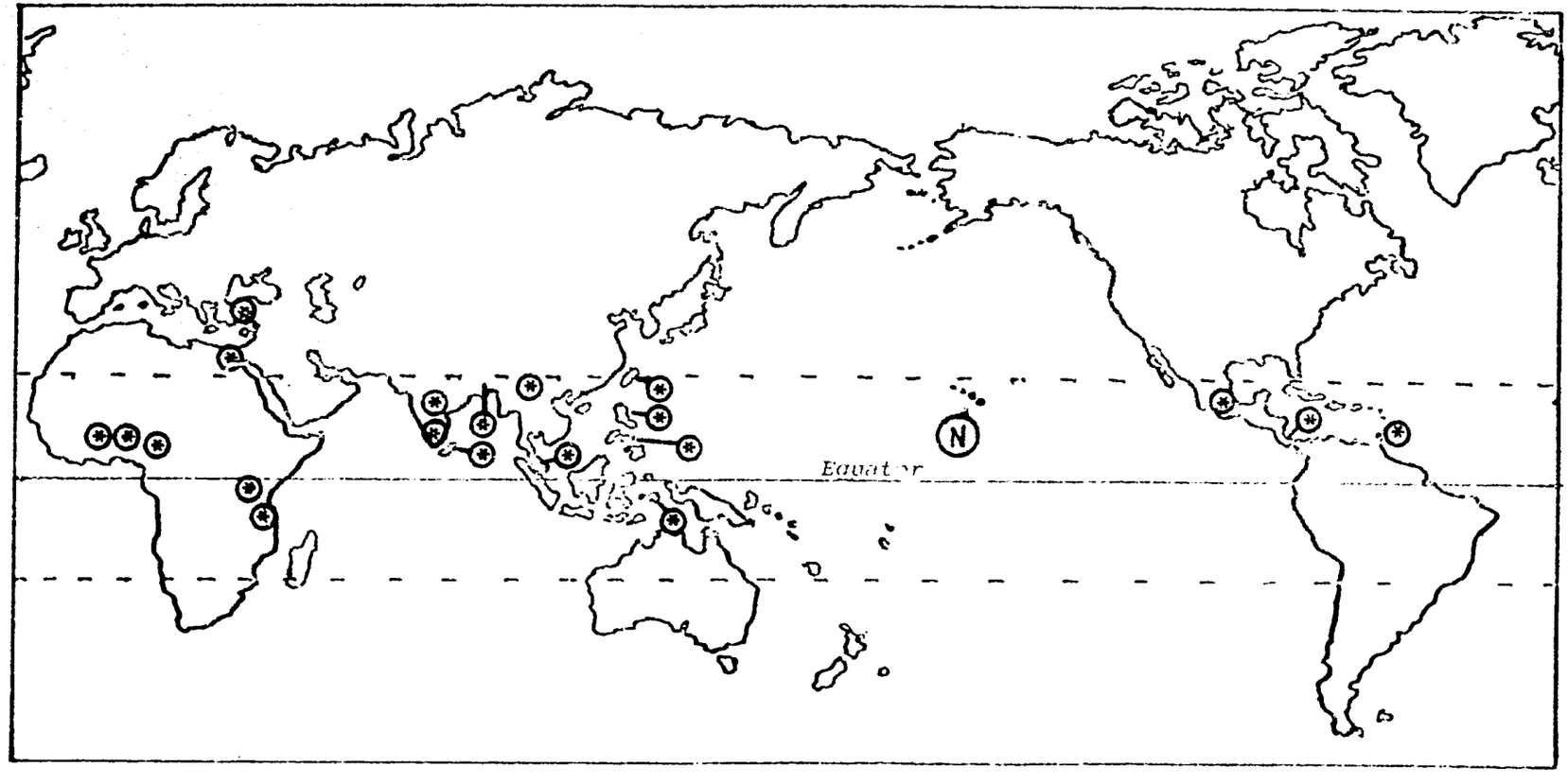
2) Mrs. Sheila May: Ecology of *Rhizobium* associated with *Vicia sativa* and *Cicer arietinum* (see Table 1).

3) Mr. Paul Singleton: *Rhizobium* affinities of tropical peanuts and significance of ineffective nodulation in soybean (see Table 1 and Section Ib-B-1).

4) Mr. Paul Woomer: Nitrogen fixation in the tuber-forming legumes (see Table 1). Mr. Woomer also presented a paper at the Winged Bean Seminar sponsored by the Asia Foundation in Manila, January 9-12, 1978 (Appendix III).

Two other graduate students who were affiliated with the project completed their Ph.D. dissertations during the year as follows:

1) Flemming I. Eriksen (Hawaii Agric. Expt. Station Graduate Research Assistant): "The effect of shading on morphology, yield, and nitrogenase activity of grain legumes and tropical forage legumes and grasses". Completed December, 1977. This dissertation embodies three manuscripts which are publishable with minor revisions.



Home Countries of NifTAL *Rhizobium* Technician Trainees

Bangladesh	Indonesia	Nigeria	Taiwan
Egypt	Kenya	Panama	Tanzania
Ghana	Malaysia	Philippines	Thailand
India	Mexico	Sri Lanka	Trinidad
			Turkey

2) Yukio Kitamura (East-West Center Graduate Research Intern): Two experiments conducted at NifTAL incorporated into his thesis will be published separately. One manuscript is in preparation: "Legume growth and nitrogen fixation as affected by plant competition for light and for soil nitrogen" by Y. Kitamura, A. B. Guevarra, and A. S. Whitney.

PURPOSE IV. Development of a Network of Collaborators

A. Working Objectives

1) To exchange *Rhizobium* germplasm and related documentation.

2) To assemble, index and exchange research information and published work concerning *Rhizobium* and inoculant technology.

3) To encourage cooperative collection, identification, and testing of *Rhizobium* strains.

B. Progress to Date

1) Exchange of *Rhizobium* Germplasm and Related Documentation: (Microbiology staff)

The sections in this report covering the *Rhizobium* Strain Collection (p. 7), and Utilization of Results (p. 43) describe the exchange of germplasm and dissemination of results of the work conducted by NifTAL. These activities constitute an integral part of the general exchange and collaboration function of the project, and contribute signficantly to the span of exchange contacts maintained by the project.

2) Literature Exchange: (Bose)

From an initial data base of 715 computer-generated references to published documents relevant to the work of the NifTAL Project, a document file with more than 2,200 entries has been compiled. Exchange correspondence with scientists in 46 countries has produced a continuously growing number of exchange relationships. A selective bibliography with annotations (1181 entries) is ready for publication, and will be distributed to the entire NifTAL mailing list in October. Approixmately 300 copies of the 1976 workshop *Proceedings* (486 pp. total) were distributed to our established contacts and to an overwhelming number of requesters. The demand has far exceeded the supply and a reprinting is being negotiated. The worldwide demand for such publications is clearly indicated by the volume of requests received.

3) Cooperative Testing Programs: (Whitney, et al.)

A good foundation for cooperation with other institutions, agencies and individuals was laid during the August, 1976 workshop on "Exploiting the legume/*Rhizobium* symbiosis in tropical agriculture" sponsored by the NifTAL Project. The 55 pariticpants came from 14 nations, plus Puerto Rico and the United States. The discussion sessions addressed some of the problems encountered in carrying out *Rhizobium* research, with strategies for their solution. Major national and international research centers and agencies represented included:

AVRDC (Taiwan, Republic of China)
CIAT (Colombia)
Central Agricultural Station (Guyana)
CSIRO (Australia, two divisions)
Department of Agriculture (Australia)
EMBRAPA (Brazil)
ICRISAT (India)
IITA (Nigeria)
Instituto Superiore Agropecuareo (Mexico)
Inter American Institute of Agricultural Sciences (Dominican Republic)
INTSOY (U.S.-based)
Lembaga Biologi Nasional (Indonesia)
Rothamsted Experimental Station (England)
USAID (Washington, D. C., USA)
USDA (Beltsville, Maryland, USA)

Six U.S. and five foreign universities were also represented, as were inoculant laboratories in India, Uruguay and the United States. Students attending the workshop sessions came from Japan, Malaysia and Thailand. Cooperative contacts with ten additional nations have been made through the four NifTAL technician training courses.

Together, these workshop participants, students and trainees form the nucleus of a "common interest" network which now reaches almost every nation which could benefit from an exchange relationship with NifTAL. The literature exchange network (see p. 40) and the germplasm exchange program (p. 40), added to the above contacts, place the NifTAL Project in a position to be of great service to the world scientific community.

These working relationships have not been organized into a formal network during the initial 3-year phase of the project. However, planning is well underway for a formal network of cooperators.

1) Dr. A. S. Whitney travelled to Iowa State (Ames), USAID (Washington, D. C.), and North Carolina State University (Raleigh) in June, 1977 to confer with AID Regional Bureaus and contractors regarding procedures for developing such a network. Contacts included Dr. Keith Whigham, formerly INTSOY Field Trial Coordinator (presently at Iowa State Univ.); representatives of the Asian, Latin American and African Bureaus; and Dr. Joe Sasser and others of the International Meloidogyne Project headquartered at NCSU.

2) Further planning was done on June 1-2, 1978 during a visit by Project Monitor L. R. Frederick to Maui. On the basis of these meetings, the groundwork has been laid for establishing a formal network of collaborating scientists throughout the tropics. A design workshop has been therefore scheduled for January 15-19, 1979, with regional workshops to be held later in 1979 and early 1980. Through these meetings, the network will be formally organized. It will involve a system of grants to collaborators and provision of bibliographic and serological services. Senior NifTAL representatives will schedule visits to the collaborators to coordinate the activities of the network.

DISSEMINATION AND UTILIZATION OF RESULTS

I. Dissemination of *Rhizobium* Strains: (Reyes/Somasegaran/Kato)

In addition to the strains distributed in earlier years (over 22 research workers in 15 tropical countries), *Rhizobium* cultures were distributed to 22 workers in 17 countries in 1977-78 as shown in Table 11 for a total of 184 cultures. (See map, p. 8)

Table 11. Recipients of *Rhizobium* cultures from the NifTAL collection by countries.

Country	No. of Recipients	Country	No. of Recipients
Australia	1	Mexico	1
Bangladesh	2	Nigeria	1
Brazil	1	Panama	1
Canada	2	Philippines	2
India	2	Sri Lanka	1
Kenya	2	Taiwan	1
Liberia	1	Trinidad	1
Malaysia	1	Turkey	1
		Zambia	1

Negotiations with the World Data Center for Microorganisms (Univ. of Queensland) to produce a catalogue jointly with other tropically-oriented collections were stalled when a) the WDCM lost their key systems specialist and b) the other collections were slow to submit the documentation.

Thus the NifTAL Project issued its own abbreviated catalogue in December, 1977 and circulated it to those known to be interested. The catalogue was revised slightly in April (Appendix I), but was not widely circulated, awaiting the results of additional screening trials and a major revision scheduled for September, 1978.

II. Dissemination of Information Concerning NifTAL Services and Research Findings

A. Sixth American *Rhizobium* Conference -- August 29 to September 2, 1977.

The project was represented by Dr. Bohlool, Mr. Keyser, Dr. Reyes, Dr. Somasegaran, and Dr. Whitney (part-time). Four papers were presented as follows (see Abstracts, Appendix IIIc):

1) Characterization of rhizobia in an acid environment, by H. H. Keyser and D. N. Munns.

2) Cross infection in the *Vigna* and *Arachis* spp. by cowpea-type rhizobia, by V. G. Reyes and P. L. Nakao.

3) A simple shielding method for avoiding excessive root-zone temperatures in container-grown legumes in the glasshouse, by Padmanabhan Somasegaran, R. W. Weaver, and L. W. Barnes.

4) NifTAL's role in increasing the exploitation of nitrogen fixation by tropical agricultural legumes, by A. S. Whitney and B. B. Bohlool.

Informal discussions were also held with a number of other *Rhizobium* workers concerned with tropical legumes such as Dr. P. Graham and J. Halliday of CIAT, Dr. Roger Smith (UPR), Dr. Steward Smith (INTSOY) and others.

B. Annual Meeting of the American Society of Agronomy -- November 13-17, 1977.

Due to the proximity of the meetings in Los Angeles, NifTAL was represented, by Dr. A. B. Guevarra, Dr. K. R. Stockinger, and graduate students Flemming Eriksen and Kenneth Cassman. Dr. Guevarra and Mr. Eriksen presented papers as follows:

1) Light intensity effects on yield and nitrogenase activity of three grain legumes, by F. I. Eriksen and A. S. Whitney.

2) Yield of *Leucaena leucocephala* and its nitrogen contributions to intercropped maize, by A. B. Guevarra, Y. Kitamura, A. S. Whitney, and J. R. Thompson.

C. Workshop/Seminar on the Development of the Potential of the Winged Bean -- Los Banos and Manila, January 9-14, 1978.

NifTAL was represented by Graduate Research Assistant Paul Woomer who presented the following paper: "Winged bean investigations at NifTAL: Observational garden, *Rhizobium* strain testing, and response across a liming gradient" by P. Woomer, A. B. Guevarra, and K. R. Stockinger (Appendix IIIb) Mr. Woomer subsequently travelled to Palawan where he was able to observe and make suggestions regarding development programs involving winged bean, leucaena, and other legumes.

D. Other Manuscripts Prepared

1) Tolerance of soil acidity in symbiosis of *Vigna radiata* with rhizobia, by D. N. Munns, H. H. Keyser, V. W. Fogle, J. S. Hokenberg, T. L. Righetti, D. L. Lauter, M. G. Zarong, K. L. Clarkin, and K. W. Whitacre. May, 1978. Submitted to Agronomy Journal.

2) A low-cost system for circulating nutrient solutions in pot studies, by A. B. Guevarra, Y. Kitamura, and A. S. Whitney. Submitted to Agronomy Journal.

3) The legume-*Rhizobium* symbiosis in tropical agriculture. A bibliography with annotations. This volume is nearing completion by Project Bibliographer John Bose. It is scheduled for publication and distribution by NifTAL in September, 1978.

4) *Rhizobium* strain catalogue (see previous section).

E. Travel by Project Staff

1) Dr. A. S. Whitney travelled for approximately 3 weeks in India and Sri Lanka in September, 1977. He visited the offices of USAID and Indian Council of Agricultural Research in New Delhi, and visited research centers in New Delhi (I.A.R.I.), Punjab, Haryana, M.P., A.P. (2 including ICRISAT), Karnataka (3), Tamil Nadu (2), and Sri Lanka. Special emphasis was placed on coordination with the INTSOY program in Sri Lanka, the All-India Coordinated *Rhizobium* Strain Trials, and ICRISAT. Private inoculum producers were visited at Bangalore (Karnataka) and Jabalpur (M.P.). Also one former trainee was visited, and the laboratories of two other NifTAL trainees were visited in their absence. Numerous contacts were made with Indian workers, many of which are being nurtured through continuing correspondence.

2) Dr. V. G. Reyes visited Kenya, Egypt, Iran, Thailand, the Philippines and Taiwan, in January, 1978 with the major objective of follow-up visits to former NifTAL trainees (except Iran). He also collected a large number of *Rhizobium* cultures, root nodules, and legume seeds for use in the *Rhizobium* testing program, and visited additional research centers where valuable contacts were made for future cooperative programs.

F. Visitors

1) Dr. P. S. Nutman, Head, Microbiology Department, Rothamsted Experiment Station -- August 12-13, 1977.

2) Dr. R. L. Knowles, Forest Research Insitute, Rotorua, New Zealand -- October 3, 1977.

3) Dr. James Litsinger, Cropping Systems Program, IRRI, Los Banos, P.I. -- October 13-14, 1977.

4) Alfonso Puyat, Makati, P. I. -- October 17, 1977.

5) H. G. Park, AVRDC, Taiwan -- November 22, 1977.

6) A. G. Norman, USAID Consultant, Ann Arbor, Michigan -- January 5, 1978.

7) M. E. Raymundo, Philippine Director, Benchmark Soils Project -- January 11-12, 1978.

8) Dr. B. Mosse*, Mycologist, Rothamsted Experiment Station -- February 20, 1977.

9) Dr. E. C. Tigchelaar, Purdue University -- March 3, 1978.

10) Mr. R. Gault*, CSIRO, Canberra, Australia -- March 28-29, 1978.

11) Dr. John Sites, University of Florida -- June 22, 1978.

12) Prof. M. Dilworth*, Murdock Univesity, Western Australia -- June 26-27, 1978.

13) Creighton Miller, Texas A & M University -- June 26, 1978.

14) Dr. R. Morris, IRRI, Los Banos, P. I. -- June 28-29, 1978.

Visitors to Davis Only

15) Yenchai Vasuvat, Thai. Ministry of Agriculture -- October, 1977.

16) Dr. P. L. Chatel, Western Australia Department of Agriculture -- August, 1977.

17) Dr. Joe Marlow, Celpril Industries -- September, March and May.

*Visitors to both Hawaii and Davis facilities.

EXPENDITURES, OBLIGATIONS, AND CONTRACTOR RESOURCES

I. Expenditures and Obligations

	June 1, 1978 to June 30, 1978 (13 mos.)*	Total to June 30, 1978
Salaries & Wages	$191,998	$378,300
Fringe Benefits	31,614	61,450
Overhead	57,336	110,635
Consultants (excluding consultant travel)	2,085	7,885
Travel & Transportation	23,893	50,537
Publications	---	377
Equipment, Supplies & Services	50,184	207,934
Vehicles (lease)	4,822	9,458
Participant Costs	17,613	46,523
Subcontract	10,000	78,906
	$389.545	$952,005

II. Estimated Expenditures and Obligations by Outputs

Culture Collection	$ 62,330
Screening & Field Testing of Cultures	120,760
Maximizing N Contribution	116,860
Training Course	66,220
Establish Network (including travel, etc.)	23,375
	$389.545

*Including expenditure of most of the funds previously reported as encumbered as of May 31, 1977. Net additional expenditures and obligations for the period June 1, 1977 to June 30, 1978 = $363,668 ($952,005 - $588,337).

III. Contractor Resources

During the third year of the contract several changes were made which gave increased capability for carrying out the contract program.

In December, 1977, Mr. John Bose was hired as project bibliographer on a half-time basis. Mr. Bose had previously been assisting the project on a temporary less-than-half-time appointment, and this change has thus improved the project capability in serving as an information base for nitrogen fixation by tropical agricultural legumes. Mr. Bose serves the NifTAL staff, NifTAL trainees and graduate students, as well as the informal common-interest network associated with the project.

Also, in December, Mr. James Kato was hired as associate curator of the *Rhizobium* collection. He is thus responsible (under Dr. Somasegaran) for the day-to-day maintenance, authenticating, storage, and culturing of strains for distribution to project staff and to workers throughout the tropics. This has greatly improved the project's capability to maintain the integrity of the collection and the response time for requests for cultures without increasing the demands on the research and training staff.

Improvements were made to the greenhouse facilities to provide suitable growing conditions during hot weather. This was achieved by a) providing shields for each Leonard jar assembly or pot; b) providing shade cloth to reduce the radiation load during midday; c) using light-colored or reflective material on the pot surface to reflect radiation; and d) ducting the air flow beneath the benches for more direct cooling. All of these improvements are applicable to similar conditions in tropical developing countries.

Arrangements were made to conduct additional field experiments on sites operated by the Hawaii Agricultural Experiment Station and the Benchmark Soils Project, thereby expanding the capability of the project to carry out field trials on different soil series.

PLAN OF WORK

July 1, 1978 to June 30, 1979

I. *Rhizobium* Collection and Screening to Identify Superior Strains

A. Identify one or more effective strains for 12 or more of the following species identified by the NAS as legumes of under-utilized potential, either by screening at NifTAL or by requesting previously-tested strains from other laboratories.

1. Mexican yam bean *(Pachyrrhizus erosus)*
2. African yam bean *(Sphenostylis stenocarpa)*
3. Bambarra goundnut *(Voandzeia subterranea)*
4. Lablab bean *(Lablab purpureus)*
5. Moth bean *(Vigna aconitifolia)*
6. Rice bean *(V. umbellata)*
7. Tepary bean *(Phaseolus acutifolius)*
8. Scarlet runner bean *(P. coccineus)*
9. Jackbean *(Canavalia ensiformis)*
10. Swordbean *(C. gladiata)*
11. Lima bean *(P. lunatus)*
12. Tarwi *(Lupinus mutabilis)*
13. Tamarind *(Tamarindus indicus)*
14. *Acacia albida*
15. *Prosopis chiliensis*
16. *Acacia auriculiformis*
17. *Albizia falcata*
18. *Sesbania grandiflora*
19. Sunnhemp *(Crotolaria juncea)*

B. Identify one or more superior strains for four or more of the following pasture legumes:

1. Greenleaf desmodium *(D. intortum)*
2. Kaimi desmodium *(D. canum)*
3. *Desmodium heterophyllum*
4. *D. ovalifolium*
5. Carpon desmodium *(D. heterocarpon)*
6. *D. triflorum*
7. *Neonotonia wightii (=Glycine wightii)*
8. *Calopogonium coeruleum*
9. *Vigna parkerii*

C. Obtain promising *Rhizobium* strains (and seeds of associated legume where appropriate) from CSRC N-fixation grant recipients.

D. Continue testing of newly-acquired *Rhizobium* strains for target grain legumes as indicated by 1) number of new acquisitions and 2) adequacy of previously tested strains.

E. Confirm the effectiveness of approximately twenty mutant (antibiotic-resistant) variants of stock cultures.

F. Produce eight or more antisera to strains presently being used for class or field testing purposes, and check titers on all presently-held anitsera.

G. Evaluate the variation in effectivity among colonies within two or more standard *Rhizobium* strains, and determine the extent to which the more highly effective variants maintain their effectiveness in subsequent generations.

H. Determine the infectivity of three elite *Rhizobium* strains from the NifTAL Collection when placed in competition with native rhizobia, using pots of soil from 3-5 selected locations including Benchmark Project sites.

I. Circulate a revised catalogue to workers throughout the tropics.

J. Carry out field experiments to a) determine the response to inoculum, and b) compare the effectiveness of two or more strains at Branch Station sites on Maui and Oahu using at least one grain legume and one pasture legume at each site.

II. Inoculation Methodology

A. Compare the survival of selected *Rhizobium* strains in peat carrier subjected to 28, 37, and 44°C.

B. Compare the survival of one or more *Rhizobium* strains in peat carriers which are maintained under 1) high moisture conditions; 2) low moisture (by addition of modified starch "super-slooper"); and 3) standard moisture levels (40-45% of MCH).

C. Carry out initial feasibility tests on the effects of possibly-beneficial fungi *(Penniciillium, Rhizopus, Fusarium)* on the growth and survival of *Rhizobium* in peat carrier.

D. Evaluate the feasibility of porous volcanic cinder (granule size) as a possible inoculum carrier.

III. Maximizing N-fixation Through Management

A. Determine the effect of starter nitrogen on the growth and N-fixation of soybean (2 cv.) and cowpea (1 cv.) in the greenhouse.

1. Effects of nitrate N at planting time on infection and nodule formation.
2. Effects of nitrate N after nodule formation on subsequent rates of growth and N-fixation.

B. Initiate a field experiment to confirm the results obtained above.

C. Determine the effects of calcium on the rates of growth and N-fixation of *Leucaena leucocephala* and *Neonotonia wightii (=Glycine wightii)*; using both symbiotic and non-symbiotic (N-fertilized) treatments in greenhouse pots.

D. Develop a non-destructive assay for estimating N-fixation during later stages of growth of grain legumes, and evaluate varietal variation for N-fixation during pod-filling for two grain legume species (e.g. *Phaseolus vulgaris*).

IV. Training in *Rhizobium* Technology

A. Carry out detailed planning and preparations for conducting a 6-week training course to be held at Nairobi in July/August 1979 with co-sponsorship of the University of Nairobi.

V. Network Establishment

A. Conduct a planning workshop in January, 1979, bringing appropriate scientists and adminstrators together for the purpose of making recommendations for the: 1) scope; 2) general methodology; 3) financial and other supportive arrangements: 4) linkages and coordination, and 5) evaluation procedures for the proposed network.

B. Carry out detailed advance planning for additional regional workshops to be held in 1979/80 at 2-3 locations in Asia.

BUDGET FORECAST
, 1978 to June 30, 1979

A. Expenditures According to Inputs:

Salaries and Wages	$228,100
Fringe Benefits	40,800
Consultants	4,000
Network Costs	10,000
Travel and Subsistence	19,000
Participant Costs	18,000
Publications	3,000
Equipment and Supplies	60,000
Subcontract (U.C. - Davis)	35,250
Vehicle Lease	6,200
Indirect Costs	72,262
Total	$496,612

B. Expenditures According to Outputs:

Rhizobium Collection	$ 49,700
Selection of Superior Strains	124,350
Network of Field Testing	39,800
Inoculation Methodology	74,600
Legume N Contribution to Cropping Systems	149,200
Training Program	58,962
Total	$496,612

Appendix I.

CATALOGUE OF STRAINS

FROM THE

NIFTAL RHIZOBIUM COLLECTION

(ABBREVIATED)

APRIL, 1978

NIFTAL PROJECT

UNIVERSITY OF HAWAII
COLLEGE OF TROPICAL AGRICULTURE
DEPARTMENT OF AGRONOMY
AND SOIL SCIENCE

U.S. AGENCY FOR INTERNATIONAL
DEVELOPMENT
TECHNICAL ASSISTANCE BUREAU
OFFICE OF AGRICULTURE

MAILING ADDRESS:

P. O. BOX 'O', PAIA, MAUI, HAWAII, 96779, U.S.A.

LIST OF ABBREVIATIONS

A. Source Laboratories and Countries

Allen	O. N. Allen Collection, Madison, Wisconsin, U.S.A. (Mrs. Ethel Allen)
Austr	Australia
Bacto	Bactogin Laboratories, Jabalpur, India (V. P. Sahni)
Cambr.	Cambridge University, United Kingdom
CIAT	Centro Internacional de Agricultura Tropical, Cali, Colombia (P. H. Graham)
HAU	Haryana Agricultural University, Hissar, Haryana, India
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, A.P., India (P. J. Dart)
INRA	Laboratorie de Microbiologie des Sols, INRA, Dijon, France (M. Obaton)
INTA	Instituto de Microbiologia e Industria Agropecuarias, INTA, Buenos Aires, Argentina
Isr-MA	Legume Inoculation Division, Ministry of Agriculture, Israel (J. Schiffman)
NifTAL	NifTAL Project, University of Hawaii, Paia, Hawaii
Nit	Nitragin Company, Milwaukee, Wisconsin, U.S.A. (J. C. Burton)
Phil	Philippines
RIO(Can)	Research Institute, London, Ontario, Canada
Rhod	Rhodesia
Roth	Soil Microbiology Department, Rothamstead Experiment Station, Harpenden, Herts, U.K. (P. S. Nutman)
SPRL	Soil Productivity Research Laboratory, Marandellas, Rhodesia (M. R. Purdom)
S&LR	State Organization for Soil and Land Reclamation, Baghdad, Iraq (Y. A. Hamdi)
Syd Univ	Department of Microbiology, University of Sydney, Sydney, Australia (J. M. Vincent)
Thai	Thailand
Thai-DA	Bacteriology and Soil Microbiology Branch, Division of Pathology, Department of Agriculture, Bangkok, Thailand (Y. Vasuvat)
UASB	Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, India (R. B. Patil)
UFRGS	Facultad Agronomia, UFRGS, Porto Alegre, Brazil (J. R. Jardim Freire)
UH	Department of Microbiology, University of Hawaii, Honolulu, Hawaii, U.S.A. (B. B. Bohlool)
UMKL	Department of Genetics and Cellular Biology, University of Malaya, Kuala Lumpur, Malaysia
Univ Minn	Department of Soil Science and Microbiology, University of Minnesota, St. Paul, Minnesota, U.S.A. (G. Ham and E. L. Schmidt)
Univ Wis	Department of Microbiology, University of Wisconsin, Madison, Wisconsin, U.S.A. (W. J. Brill)
UPLB	Department of Soils, University of the Philippines, Los Baños, Laguna, Philippines (S. Tilo)

USDA	Cell Culture and Nitrogen-Fixation Laboratory, U. S. Department of Agriculture, Agricultural Research Center, Beltsville, Maryland, U.S.A. (D. F. Weber)
US-Miss	Mississippi, U.S.A.
US-Penn	Pennsylvania, U.S.A.
US-Va	Virginia, U.S.A.
US-Wis	Wisconsin, U.S.A.

B. Legume Species

Arh	*Arachis hypogaea* (peanut, groundnut)
Cac	*Cajanus cajan* (pigeon pea, red gram)
Cag	*Canavalia gladiata* (sword bean)
Cam	*Calopogonium muconoides* (calopo)
Cep	*Centrosema pubescens* (centro)
Cia	*Cicer arietinum* (chick pea, garbanzo, gram)
Dec	*Desmodium canum* (kaimi clover)
Dei	*Desmodium intortum*
Dex	*Desmodium species*, wide spectrum
Dol	*Dolichos lablab* (=*Lablab purpureus*) (hyacinth bean)
Glm	*Glycine max* (soybean)
Lee	*Lens esculenta* (or *L. culinaris*) (lentil)
Lel	*Leucaena leucocephala* (koa haole, ipil-ipil)
Maa	*Macroptilium atropurpureus* (=*Phaseolus atropurpureus*) (siratro)
Pae	*Pachyrrhizus erosus* (Mexican yam bean)
Pha	*Phaseolus acutifolius* (tepary bean)
Phl	*Phaseolus lunatus* (lima bean)
Phv	*Phaseolus vulgaris* (common bean, garden bean)
Pst	*Psophocarpus tetragonolobus* (winged bean)
Puj	*Pueraria javanica* (tropical kudzu)
Syg	*Stylosanthes guyanensis* or *S. fruticosa* (stylo)
Syx	*Stylosanthes* spp, wide spectrum
Vif	*Vicia faba* (faba bean, horse bean)
Via	*Vigna angularis* (adzuki bean)
Vim	*Vigna mungo* (=*Phaseolus mungo*) (black gram)
Vir	*Vigna radiata* (=*Phaseolus radiata*, *aureus*) (mungbean, green gram)
Viu	*Vigna unguiculata* (=*V. sinensis*, cowpea; and *V. sesquipedalis*, yard-long bean)
Vos	*Voandzeia subterranea* (Bambarra groundnut)

C. Rhizobium Growth Characteristics

Ac	Acid producer in YMA
Al	Alkaline producer in YMA
F	Fast grower (<5 days)
S	Slow grower (≥5 days)

HOST-STRAIN CROSS INDEX OF SELECTED STRAINS FROM THE NIFTAL RHIZOBIUM COLLECTION

HOST LEGUME	RECOMMENDED STRAINS	OTHER EFFECTIVE STRAINS*
Target Grain Legumes		
Arachis hypogaea (Peanut)	11, 98. 169, 236, 295	175, 176, 177, 388, 440, 464, 465, 466, 481 482, 483
Cajanus cajan (Pigeon pea)	255, 569, 579	191, 234, 256, 570, 646
Cicer arietinum (Chickpea)	385, 480, 619, 620, 621, 622, 623	669, 677, 678, 679
Dolichos lablab (Hyacinth bean)	169, 197, 309, 310	88, 222, 230, 237, 310, 490, 568, 574, 580, 670, 671, 672
Glycine max (Soybean)	102, 103, 183, 206, 216, 377, 378, 379, 411, 415, 629	184, 185, 410, 435, 630, 631, 632, 633, 649, 650
Lens esculenta (Lentil)	634, 638, 639, 640	
Phaseolus vulgaris (Garden bean)	182, 241, 460, 462, 616, 659, 660, 661, 662, 663, 664, 665	490, 613
Vigna mungo (Black gram)	174, 309, 438, 570 684	495, 574, 673
Vigna radiata (Mungbean)	169, 207, 303, 420, 425, 438, 440, 441, 442, 463	495, 570, 673, 674, 675, 676
Vigna unguiculata (Cowpea)	11, 163, 169, 174, 176, 189, 235, 309, 641	310, 568, 581, 668
Voandzeia subterranea (Bambarra groundnut)	169	

HOST-STRAIN CROSS INDEX OF SELECTED STRAINS FROM THE NiFTAL RHIZOBIUM COLLECTION

HOST LEGUME	RECOMMENDED STRAINS	OTHER EFFECTIVE STRAINS*
Target Pasture Legumes		
Calopogonium muconoides	651	
Centrosema pubescens	651, 652, 653, 654, 655	392, 467, 471
Desmodium intortum	309, 569, 668	564, 570, 573, 578, 667
Desmodium uncinatum	569	570, 571, 574, 682
Glycine wightii		684
Leucaena leucocephala	21, 82, 590, 603	582, 583, 593, 602
Macroptilium atropurpureus	309	
Pueraria javanica	647, 651	
Stylosanthes guyanensis	658, 666	403, 404, 405, 406, 564 565, 575, 682
Stylosanthes hamata	658, 666	403, 404, 405, 406, 564, 565, 575, 682
Target Minor Legumes		
Canavalia gladiata	643	
Pachyrrhizus erosus	656, 657	
Phaseolus acutifolius	642, 644, 648	
Phaseolus lunatus	22	
Psophocarpus tetragonolobus	281, 309, 642, 643, 648, 651, 656	
Vicia faba	167	
Vigna angularis	645	

*Not all of these strains are documented in the following section or at WDCM.

SELECTED STRAINS FROM THE NifTAL RHIZOBIUM COLLECTION

TAL#	SOURCE:DESIG:PRIOR DESIGNATION	ORIGIN	EFFECTIVENESS	GROWTH ON YMA	COMMENTS
11	NifTAL	Hawaii (1975)	Arh*, Viu*, Vir	S/Al	
21	Nit:94A1	US-Wis (19747)	Lel	F/Ac	
22	NifTAL	Hawaii (1975)	Phl*	S/Al	
82	NifTAL	Hawaii (1975)	Lel*	F/Ac	
98	CIAT:239	Colombia	Viu, Arh*	S/Al	
102	USDA:110	US-Florida	Glm*	S/Al	
103	Nit:61A118		Glm*	S/Al	
165	NifTAL	Nigeria (1975)	Viu*	S/Al	
167	Nit:175F1	Holland	Vif*	F/Ac	
169	Nit:176A22	US-Wis (1966)	Viu*, Vir*, Arh*	S/Al	Mod. acid tolerant.
174	Nit:176A32	Nigeria (1975)	Viu*, Vim	S/Al	
175	Nit:8A11		Arh	S/Al	
176	Nit:8A14	Us-Va (1974)	Arh	S/Al	
177	Nit:8A16	US-Texas (1975)	Arh	S/Al	
182	NifTAL	Hawaii (1976)	Phv*	F/Ac	
183	Nit:61A76	US-Mis (1953)	Glm*	S/Al	
184	Univ Wis:SM-31	US-Wis	Glm	S/Al	
185	Univ Wis:SM-35	US-Wis	Glm	S/Al	
189	Nit:176A31	Nigeria (1975)	Viu*	S/Al	
191	Nit:21A8	Nigeria (1975)	Cac	S/Al	
197	Nit:42B3	Brazil (1974)	Dol*	S/Al	
206	NifTAL	Thailand (1976)	Glm*	S/Al	
207	NifTAL	Thailand (1976)	Viu, Vir*	S/Al	
216	NifTAL	Thailand (1976)	Glm*	S/Al	

SELECTED STRAINS FROM THE NifTAL RHIZOBIUM COLLECTION

TAL#	SOURCE:DESIG:PRIOR DESIGNATION	ORIGIN	EFFECTIVENESS	GROWTH ON YMA	COMMENTS
235	NifTAL	Hawaii (1976)	Viu*	S/Al	
236	NifTAL	Hawaii (1976)	Arh*	S/Al	
241	NifTAL	Hawaii (1976)	Phv*	F/Ac	
255	NifTAL	Hawaii (1976)	Cac*	F/Ac	
281	NifTAL	Hawaii (1976)	Dec*	S/Al	
295	NifTAL	Hawaii (1976)	Arh*	S/Al	
303	NifTAL	Phil (1976)	Viu, Arh, Vir*	S/Al	
309	Syd Univ::CB756		Viu*, Arh*, Vir, Dol*, Vim	S/Al	
310	Syd Univ::CB1024		Dol*, Viu	S/Al	Acid sensitive.
377	Univ Minn::USDA-138		Glm*	S/Al	
378	Syd Univ::CC709		Glm*	S/Al	
379	Syd Univ::CB1809	(=USDA-136b)	Glm*	S/Al	
385	Syd Univ::CB1189		Cia	----	
388	Bacto:173/BLR.	India (1976)	Arh	S/Al	
410	Thai-DA:THA2	Thailand	Glm	S/Al	
411	Thai-DA:THA3	Thailand	Glm*	S/Al	
415	Thai-DA:THA9	Thailand	Glm*	S/Al	
420	Thai-DA:THA301	Thailand	Vir*, Viu, Arh	S/Al	
425	Thai-DA:THA306	Thailand	Vir*, Viu, Arh	S/Al	Acid tolerant.
435	UFRGS:587	Brazil	Glm	S/Al	
438	UPLB:M3	Phil (1976)	Vir*, Vim	S/Al	
440	UPLB:M5	Phil (1976)	Vir*, Arh	S/Al	
441	UPLB:M6	Phil (1976)	Vir*	S/Al	

SELECTED STRAINS FROM THE NiFTAL RHIZOBIUM COLLECTION

TAL#	SOURCE:DESIG:PRIOR DESIGNATION	ORIGIN	EFFECTIVENESS	GROWTH ON YMA	COMMENTS
442	UPLB:M7	Phil	Vir*	S/Al	
460	S&LR:IQ403	Iraq	Phv*	F/Ac	
462	S&LR:IQ423	Iraq	Phv*	F/Ac	
463	S&LR:IQ68-5	Iraq	Vir*	S/Al	Acid sensitive.
464	Isr-MA:38b/70	Israel	Arh	S/Al	
465	Isr-MA:280A	Israel	Arh	S/Al	
466	Isr-MA:R/283A1	Israel	Arh	S/Al	
480	UASB:67	India	Cia	S/Al	
481	UASB:94	India	Arh, Viu	S/Al	
482	UASB:95	India	Arh, Vir	S/Al	
483	UASB:96	India	Arh, Vir	S/Al	
565	SPRL:411:Allen627	US-Wis (1946)	Dex, Dol, Syx	S/Al	
568	SPRL:468	Rhod (1960)	Viu	S/Al	
569	SPRL:472	Rhod (1960)	Cac*, Dex, Maa, Syg	S/Al	
570	SPRL:484	Rhod (1962)	Vim*, Cac		
574	SPRL:657	Rhod (1961)	Dol		
579	SPRL:998	Rhod (1965)	Cac*, Viu, Maa	S/Al	
590	NiFTAL	Hawaii (1977)	Lei*	F/Ac	
603	NiFTAL	Hawaii (1977)	Lei*	F/Ac	
616	CIAT:676:C2282	(=Cambr 2282)	Phv*	F/Ac	
619	ICRISAT:3827	(=Roth 3827)	Cia*	F/Ac	
620	ICRISAT:3889	(=Roth 3889)	Cia*	F/Ac	
621	ICRISAT:DNRa-1	India	Cia*	F/Ac	
622	ICRISAT:Cicer-5		Cia*	F/Ac	

SELECTED STRAINS FROM THE NIFTAL RHIZOBIUM COLLECTION

TAL#	SOURCE:DESIG:PRIOR DESIGNATION	ORIGIN	EFFECTIVENESS	GROWTH ON YMA	COMMENTS
623	ICRISAT:Ca-7	HAU	Cia*	F/Ac	
629	Allen:527:USDA-704	US-Penn (1936)	Glm*	S/Al	
630	Allen:511:USDA-500	Japan (Soy 1) (1929)	Glm	S/Al	
631	Allen:519	Hawaii (1930)	Glm	S/Al	
632	Allen:542:INTA-E41	Argentina	Glm	S/Al	
633	Allen:543:INTA-E45	Argentina	Glm	S/Al	
634	UH:B-13:NZP-5400	N. Zealand	Lee*	F/Ac	
638	UH:I-2	Hawaii (1977)	Lee*	F/Ac	
639	UH:I-7	Hawaii (1977)	Lee*	F/Ac	
640	UH:I-11	Hawaii (1977)	Lee*	F/Ac	
641	UMKL:35	Malaysia	Viu*	S/Al	
642	UMKL:36	Malaysia	Dol*, Pst*, Pha*	S/Al	
643	UMKL:25	Malaysia	Cag*, Pst*	S/Al	
644	CIAT:257:RIO-94	Canada	Pha*	S/Al	
645	UMKL:12	Malaysia	Via*	S/Al	
646	UMKL:69	Malaysia	Cac	S/Al	
647	UMKL:56	Malaysia	Puj*	S/Al	
648	UMKL:57	Malaysia	Pst*, Pha*	S/Al	
649	UMKL:52	Malaysia	Glm	S/Al	
650	UMKL:22	Malaysia	Glm	S/Al	
651	UMKL:44	Malaysia	Cam*, Cep*, Pst* Puj*	S/Al	
652	UMKL:08	Malaysia	Cep*	S/Al	
653	UMKL:47	Malaysia	Cep*	S/Al	
654	UMKL:04	Malaysia	Cep*	S/Al	

SELECTED STRAINS FROM THE NiFTAL RHIZOBIUM COLLECTION

TAL#	SOURCE:DESIG:PRIOR DESIGNATION	ORIGIN	EFFECTIVENESS	GROWTH ON YMA	COMMENTS
655	UMKL:09	Malaysia	Cep*	S/Al	
656	UMKL:82	Malaysia	Pae*, Pst*	S/Al	
657	UMKL:81	Malaysia	Pae*	S/Al	
658	CIAT:71	Colombia	Syx*	S/Al	
659	Allen:413-2	US-Wis	Phv*	F/Ac	
660	Allen:420	Chile	Phv*	F/Ac	
661	Allen:421	Chile (1963)	Phv*	F/Ac	
662	Allen:422	Chile (1963)	Phv*	F/Ac	
663	CIAT:40:Nit-127K26		Phv*	F/Ac	
664	CIAT:248	Colombia	Phv*	F/Ac	
665	CIAT:249	Colombia	Phv*	F/Ac	
666	CIAT:308	Colombia	Syx*	A/Al	
667	SPRL:471	Rhodesia	Dei, Arh	S/Al	
668	INRA:GU-1	Guadalupe	Arh, Dex, Viu	S/Al	
669	Allen:735-USDA-330		Cia	F/Ac	
684	SPRL:429	Rhodesia	Vim*	S/Al	

*Recommended strain

Appendix IIa. Performance of *Rhizobium* strains in acid soil.

Effectiveness[1]	Tolerance Rating[2]		
	0	1	2
Experiment 1: Cowpea, TVu 1190 + TVu 4557, Josephine Soil pH 4.6			
3	170	n9, m7(442)	425, 309
2	98, 207	n16, 169, 171, 174	
1	310, 463, n15, 163, 172	---	189, 209, 173
Experiment 2: Cowpea, California Blackeye 5, Goldridge Soil 4.6			
2	303	189, 309	173, 174
1.5	---	163, 171, 172	---
1	310	98, 169, 170	---
0	11, 207	---	---
Experiment 3: Mungbean, Burken and V2184, Goldridge Soil pH 5.0			
3	463	420, 209, 438, 440, 441, 443	174, 309, 425, 442
2	n16	32H1, 169, n9, 303, 421, 423, 437, 439	---
1	172, 173	11, 170, 281, n12	---
0	98, 163, 171, 189, 207, 305, 306, 310, IQ108, IQ921, h4, h7, n10, n15, n22		
Experiment 4: Peanut, Florunner, Josephine Soil pH 4.5			
2	309, 127D5	236, 425	
1	98, 170, 175, 176, 295	11	
0	174, 207, 209, 420, 422, 442		

[1] 3 = highly effective 2 cv.; 2 = highly effective one cv., moderately effective on other; 1 = moderately effective 2 cv.; 0 = ineffective on one or both cv.

[2] 0 = sensitive; 1 = moderately tolerant; 2 = highly tolerant. Ineffective strains were not rated for tolerance.

Appendix IIb. Effects of multiple passage of *Rhizobium* through nodules of homologous and non-homologous host legumes.

Prior Host Passages[1]			Dry Weight[2] in g/Pot	Percent of N Control
1	2	3		
Experiment 1: *R. japonicum* USDA 136b (=TAL 379)				
GM			5.94 a	51
GM	GM		7.96 bc	69
CP	GM		7.74 b	67
GM	GM	GM	9.11 d	79
CP	GM	GM	9.08 cd	79
Plus N	Control	(70 ppm N)	11.56 --	100
Experiment 2: Cowpea *Rhizobium*: CIAT 239 (=TAL 98)				
CP			9.05 a	69
CP	CP		8.99 a	68
PV	CP		11.08 b	84
CP	CP	CP	10.88 b	82
Plus N	Control	(70 ppm)	13.20 --	100
Experiment 3: *R. phaseoli* TAL 241				
PV			4.79 a	53
PV	PV		5.97 ab	66
CP	PV		6.94 b	79
PV	PV	PV	6.13 ab	68
Plus N	Control	(70 ppm)	9.01 --	100

[1] GM = soybean cv. Clark 63, CP = cowpea cv. California Blackeye, PV = common bean cv. Burpee tenderpod.
[2] Data within each experiment followed by the same letter are not different at approximately the 5% level of significance using the Bayes LSD test.

Appendix IIIa. Information presented at the annual meeting of The American Society for Microbiology, Las Vegas, Nevada, May, 1978.

Application of Immunofluorescence to the Selection of Highly Competitive Strains of Lentil *Rhizobium*.

S. May and B. B. Bohlool

Abstract

The ability of highly effective strains of *Rhizobium* to compete for nodulation sites on lentils was assessed by immunofluorescence. The nitrogen-fixing potential of thirty lentil rhizobia was determined by acetylene reduction. Three superior strains (Hawaii 5-0, NZP 5400, and Nitragin 128A12) were selected for competition studies. Fluorescent antibodies for these strains proved strain-specific. Various combinations of multi-strain inoculum on lentil seedlings in sterile vermiculite yielded the follwoing results: 5-0/5400, 40%/45% and 15% double infections (DI); 5-0/A12, 65%/10% and 25% DI: 5400/A12, 90%/10% and 0% DI; 5-0/5400/A12, 26%/61%/0%, and 13% DI (5-0 and A12), and no triple infections. Seeds were pelleted with the best competitors (5-0 and 5400), either singly or in combination, and grown in the field. Both strains outcompeted the indigenous rhizobia, all of the nodules being from the inoculum strain. In double-strain inoculation trials in the field, 40% of the nodules were occupied by 5-0, 40% by 5400, and 20% contained both strains.

Appendix IIIb. Paper presented by Mr. Paul Woomer at Workshop/Seminar on the Development of the Potential of the Winged Bean, Los Banos, Philippines, January, 1978.

WINGEN BEAN INVESTIGATIONS AT THE NifTAL PROJECT: OBSERVATIONAL GARDEN, RHIZOBIUM STRAIN TESTING AND RESPONSE ACROSS A LIMING GRADIENT[1]

Mr. Paul Woomer, Dr. Anacleto Guevarra and
Dr. Karl Stockinger

Abstract

Three areas of research by the University of Hawaii NifTAL Project concerning the winged bean Psophocarpus tetragonolobus are reported upon. Two plantings of winged beans were made, first 12 and then 37 additional lines are compared. The ranges of several measured attributes were: Yield of dried seeds per plant, 17 grams to 420 grams; the weight of 100 seeds, 21.9 grams to 45.6 grams; pod length from 10 cm. to 31 cm.; nitrogen content of green edible pods, 3.11% to 5.49%; pod numbers, 6 to 108 pods per plant; and shelling percentage, 45% to 60%. The outstanding varieties under Paia, Hawaii, conditions were a long podded selection from the Philippines and another from the Malaysia Agricultural Research and Development Institute.

Six cowpea-type Rhizobium strains were screened for effectiveness on winged bean (Tpt-1 via Fiji). There were no significant differences among the most effective strains. Nodule mass and acetylene reduction were significantly correlated with shoot dry matter production.

Winged beans (selection 1014, Bogor, Indonesia) were grown across a pH (lime) gradient. The soil pH levels varied from 4.6 to 6.8 across the gradient. Winged bean shoot mass increased twofold between pH 4.7 and 5.5. There were no further increases after pH 5.5. Nitrogen percentage in the tissues showed a similar trend, varying from 3.25% at pH 4.7 to 4.03% at pH 6.2.

Nodule mass and acetylene reduction activities were high at low pH levels. Evidence indicates that the winged bean is sensitive to Al^{+++} in soil solution, but that it does not have a high calcium requirement.

[1]Journal Series No. 2307 of the Hawaii Agricultural Experiment Station. This work was supported in part by the U. S. Agency for International Development (Contract ta-C-1207).

Appendix IIIc. Papers presented by NifTAL staff at the Sixth American *Rhizobium* Conference, Gainesville, Florida, August 29 - September 2, 1977.

A. Cross Infection in the *Vigna* and *Arachis* spp. by Cowpea Type Rhizobia.
V. G. Reyes and P. L. Nakao

Abstract

The promiscuity of legumes which nodulate with rhizobia of the cowpea miscellany was used to advantage in selecting effective strains of *Rhizobium* for three major food grain legumes. Thirty-eight strains of rhizobia originally isolated from cowpea (*Vigna unguiculata*), mungbean (*Vigna radiata*) and peanut (*Arachis hypogaea*) from diverse sources were cross tested on the three hosts.

Of the total 111 strain-host combinations, 30 were highly effective based on plant dry weight, acetylene reduction, ability to effectively nodulate alternate hosts, and plant color at 2 weeks after inoculation and/or during harvest. Cowpea isolates had the highest ratio of effective to ineffective strains when all three hosts were considered, followed by mungbean and peanut. Most of the effective associations, however, were formed by isolates nodulating their original host species. Cowpea was the most promiscuous and peanut the most selective for effective rhizobia.

B. A Simple Shielding Method for Avoiding Excessive Root-zone Temperature in Container-grown Legumes in the Glasshouse.
S. Padmanabhan, R. W. Weaver[1] and L. W. Barnes[1]

Abstract

Testing of *Rhizobium* strains on legume plants in the glasshouse during warm sunny weathe is often affected by high temperatures in the Leonard jars or pots. -zone temperatures, in the potting mixture commonly rise 6-10°C abo ambient. This is damaging to the germination, growth and nodulation of many legumes. Also, exposure of nodules to >30° often impairs N fixation. At the NifTAL glasshouse, the usual practice of wrapping the pots with reflective foil provided only a slight reduction in root-zone temperatures.

R. A. Date (CSIRO, Brisbane) has found that by placing cylinders of white plastic tubing covered with adjustable-aperture white plastic plates, he could shade the pots and also allow free air circulation around them. Following his example, we experimented with this technique using locally available materials. Placement of an oversized metal cylinder to surround the pot reduced the temperature in the pot to ambient or slightly below. The cylinder could be made of any convenient material but must be approximately 8cm greater in diameter than the pot to be housed. The cylinder should extend beyond the top of the pot no more than 4cm in order to avoid excessive shading of the young seedlings.

[1]Texas A & M University.

Further protection from the noon sun was obtained by placing a perforated lid (of white material) on the cylinder. The lid had small holes to allow for air exchange and a larger hole in the center for the seedlings to emerge. The lid was then removed when the seedlings were large enough to shade the surface of the potting mixture. Use of this technique has made it feasible to successfully grow vigorous legume plants even in hot sunny weather.

C. NifTAL's Role in Increasing the Exploitation of Nitrogen Fixation by Tropical Agricultural Legumes.

A. S. Whitney and B. B. Bohlool

Abstract

Since its inception in mid-1975, the NifTAL Project has developed research and training facilities and has assembled a staff to carry out a variety of activities in support of Rhizobium and legume programs in the tropics. The Rhizobium-oriented work has been conducted by Drs. T. J. Wacek, V. G. Reyes, and (more recently) Padmanabhan Somasegaran.

1. A collection of Rhizobium cultures has been assembled, including over 600 strains isolated from over 50 legume spp., mainly tropical legumes.

2. A screening program is conducted on a routine basis to check on the authenticity and evaluate the effectiveness of Rhizobium acquisitions, both on original and suspected hosts where appropriate (see abstract by V. G. Reyes). Several hundred strains have been screened to some extent to date. Selection of strains tolerant of acid-soil conditions is being done by D. N. Munns (Univ. of Calif.-Davis) under a sub-contract. Screening is also underway to select effective strains for the "Neglected Legumes" identified by the Nat'l. Academy of Sciences.

3. As this work proceeds, the collection is becoming a more valuable resource for agricultural development in the tropics. Cultures were sent to workers in 14 tropical countries last year. This aspect of NifTAL's program will undoubtedly increase upon publication of a catalogue of the collection within a few months.

4. A six-week training program was developed by consultant J. M. Vincent for NifTAL. The course has been much in demand with many more applicants than can be accommodated. To date, 17 persons serving developing countries have been trained representing Mexico, Taiwan, Philippines (3), Thailand (2), Malaysia, Indonesia, India (2), Sri Lanka, Kenya, Tanzania, Egypt, Nigeria, and Ghana. Responses from graduates indicate that the course has proved helpful in their work upon return home.

Research is also being conducted on the dynamics of N contributions by legumes to cropping systems by two professional agronomists and graduate

students. Factors being studied include shading, lime and phosphorus effects, soil nitrogen, and crop competition.

If any aspects of the NifTAL program can be of assistance to readers, enquiries are cordially invited.

D. Characterization of Rhizobia in an Acid Environment

H. H. Keyser and D. N. Munns[1]

Abstract

Members of the "cowpea miscellany" group of rhizobia show large variation in growth between strains under single or combined stress factors commonly found in acid soils. Strain tolerances to the major stresses of acidity agree well with its symbiotic performance with cowpea (Vigna unguiculata L.) in an acid soil.

Growth studies in defined broth media revealed significant differences between and within strains in ability to grow or maintain population levels under a single set of stresses. The individual stresses used were a pH of 4.6 alone; this low pH combined with a low Ca (50 μM), with low P (10 μM), or with high Mn (200 μM); and low pH with low P + high Al (50 μM). Although the more tolerant strains were able to make good growth, Mn and Al were the most severe stresses.

When five strains capable of good growth in one or more sets of the individual stresses were introduced into a medium of these combined, all showed a 100-fold decrease from their initial level, and then either maintained this level or made very slow growth over a 20 day period.

Detailed growth studies under Al stress revealed varying lag periods before net growth commenced in the Al-tolerant strains. Here also some strains were suppressed by the low level of P that was necessary to permit the existence of free Al. Further, growth from small inocula was erratic in media that provide stress, although it was very reproducible in unstressed media, suggesting the existence of variants within a single strain.

Strain tolerance ratings to the factors of acidity determined under controlled conditions agree well with the strains' symbiotic performance in an acid soil as reflected by the nodulation and growth of cowpea, however the relationship does not appear to hold with mung bean (Vigna radiata L.).

[1]University of California, Davis (under subcontract to NifTAL).