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ANNUAL REPORT

NifTAL PROJECT

July 1, 1979 - June 30, 1980

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

UH/AID Contract ta-C-1207

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

UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT

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REPORT SUMMARY

STATISTICAL SUMMARY

Title:	Maximizing Symbiotic Fixation of Nitrogen by Grain and Forage Legumes in the Tropics (NifTAL Project)
Contract:	AID/ta-C-1207
Principal Investigator:	Jake Halliday
Contractor:	University of Hawaii Honolulu, Hawaii 96822
Contract Period:	June 1, 1975 - June 30, 1981
Reporting Period:	July 1, 1979 - June 30, 1980
Total AID Funding to Date:	$2,186,518.00
Total Expenditures and Obligations through Previous Contract Year:	$1,409,304.20
Total Expenditures and Obligations for Current Year:	$642,905.95
Estimated Expenditures for Next Contract Year:	$821,041

PROJECT PERSONNEL

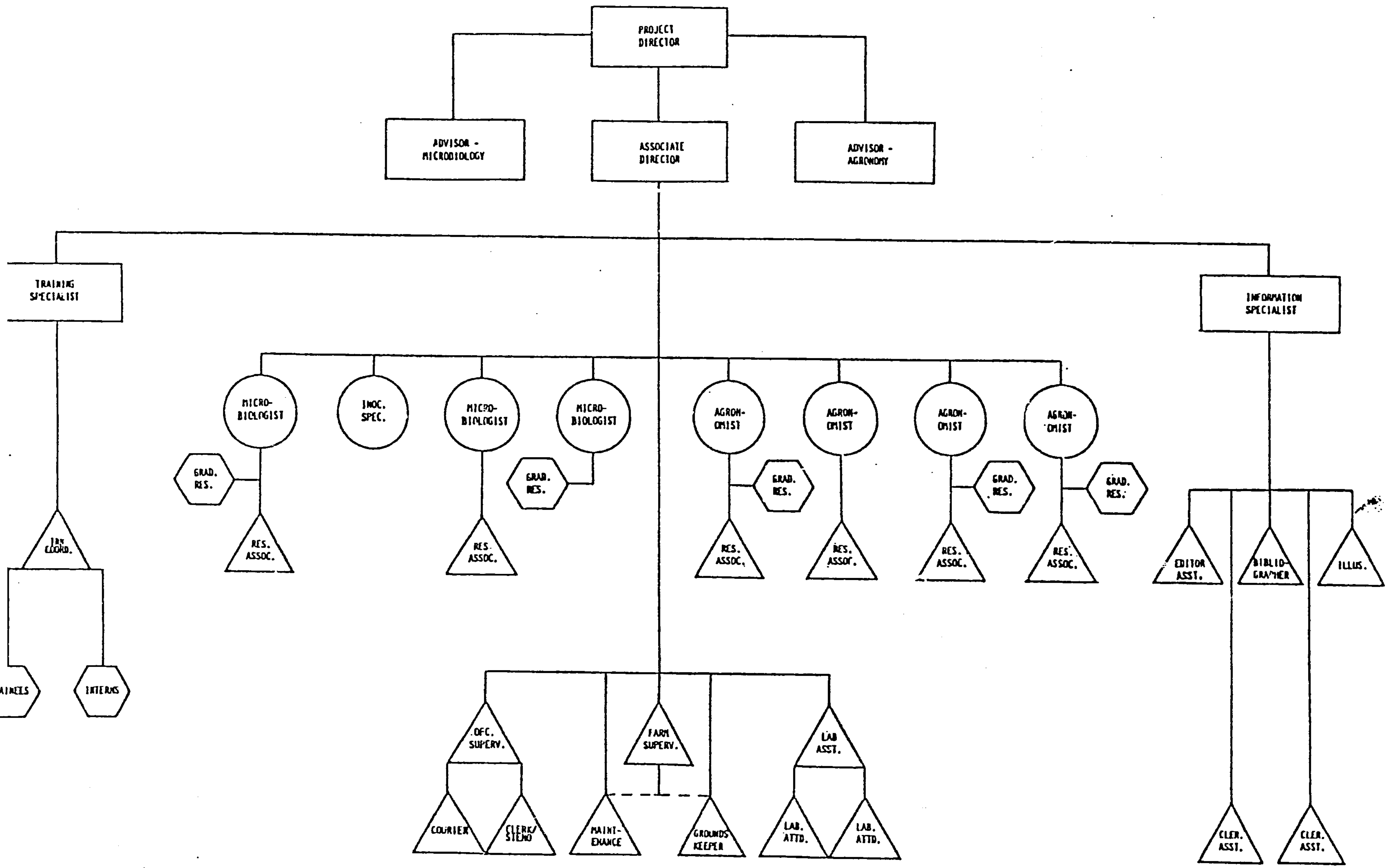
Alphabetical List of Staff:

Staff	Position
M. Akyeampong	Graduate Research Assistant
D. Billings	Research Associate
B. B. Bohlool	Advisor, Microbiology
B. Bose	Lab Assistant
J. Bose II	Bibliographer
R. Bousquet	Secretary
P. Carroll	Lab Assistant
S. Chaudhary	Graduate Research Assistant
J. De Coite	Janitor/Maintenance
R. J. Davis	Agronomist/Regional Coordinator
P. Ferguson	Courier
J. Fitzpatrick	Artist
M. Habte	Microbiologist
J. Halliday	Project Director/ Regional Coordinator
S. C. Harris	Information Specialist
H. Hoben	Research Associate
B. Kerrey	Associate Project Director
C. King	Student Assistant
J. Landa	Research Associate
K. MacGlashan	Research Associate
D. L. McNeil	Plant Physiologist
R. B. Martin	Research Associate
R. S. Mawson	Research Associate
M. Nakamoto	Student Assistant

P. Nakao	Research Associate
D. Narramore	Student Assistant
J. Rothenberg	Student Assistant
C. Shaw	Student Assistant
J. Soliven	Groundskeeper
P. Somasegaran	Soil Microbiologist/ Regional Coordinator
W. T. South	Graduate Research Assistant
K. R. Stockinger	Agronomist
A. S. Whitney	Advisor, Agronomy
G. Winn	Research Associate
J. Woods	Farm Assistant
R. Woolfenden	Research Associate
K. Zukeran	Office Manager

ORGANOGRAM

Distribution of Positions by Function



SUMMARY OF ACCOMPLISHMENTS

Since its inception in 1975, the NifTAL Project has concentrated on five program areas: building and making available a germplasm resource, training key personnel in *Rhizobium* technology, contributing research in problem areas that limit effective utilization of the legume/*Rhizobium* symbiosis, promoting an international network of BNF researchers, and disseminating BNF information among scientists throughout the tropics. The integration of these service and research programs offers germplasm, technology, and trained personnel to agencies responsible for agricultural policy in tropical countries to enable them to promote legume cropping systems.

One of NifTAL's earliest areas of emphasis was the collection and screening of rhizobia in order to develop inoculation recommendations for common and underexploited tropical legumes. As a result of this ongoing program, sizeable collections of cultures and seeds have been preserved. The Culture Collection at NifTAL now contains 1000 *Rhizobium* strains tested for tropical legumes and stored either in lyophilized ampoules for long-term storage or on ceramic beads for easy dissemination. *Rhizobium* strains are accessible to tropical researchers in the form of pure cultures or in peat-based inoculants. The antisera that have been developed for many strains are also available on request. Small quantities of seed of species that are not usually available from commercial sources can be supplied from NifTAL's collection, as well.

A major limiting factor in full utilization of the legume cropping systems is the need for scientists and technicians with the combination of agronomic and microbiological training to carry out research and extension programs. Training programs offered by NifTAL include graduate research through the University of Hawaii's Agronomy and Soil Science Department, formal six-week intensive courses in *Rhizobium* technology, and informal intern training experiences at the NifTAL laboratory. NifTAL has already trained 43 persons in addition to contributing staff time and financial support to training programs at national institutions.

Research at NifTAL backstops and improves the Project's technical assistance capacity. The major research thrusts have involved inoculant production systems and studies on maximizing the nitrogen contribution of legumes in tropical cropping systems. Studies on inoculation problems have included research on the survival potential of bacteria in peat and in the soil/root environment, simplification of procedures for inoculant production, work on improving field inoculation practices, and testing for selection of effective strains. Other ongoing research has involved the legume/*Rhizobium* symbiosis as it is affected by phosphorus level, soil pH, saline soils, soil nitrate, and water stress. The benefits of legumes to mixed or sequential cropping systems have also been evaluated regarding both cereal crops and pasture systems.

The International Network of Legume Inoculation Trials was formally initiated in January, 1979. Scientists and administrators from many national and international agencies which had previously been involved with the Project in a "common interest" network met in Hawaii and charged NifTAL with promoting a network of inoculation trials throughout the tropics. Through a series of standardized trials, NifTAL and its Cooperators are attempting to appraise, under realistic field conditions, whether the yield of tropical legumes can be increased by inoculation with effective strains of rhizobia. Ninety-five Cooperators are participants in the Network to date.

The information program has expanded in the past five years from its beginnings as a small document collection to serve Project staff. The Document Collection now contains over 1500 pieces, and through bibliographic publications and the document copying service, these items are made accessible to researchers in countries eligible for AID support. Additionally, six major NifTAL publications have been produced, two of which have been reprinted due to a constantly increasing demand. NifTAL also produces a quarterly bulletin featuring news on research in all areas of biological N fixation. This publication has a circulation of over 1000 scientists and administrators worldwide.

NifTAL has designed its programs to serve national and international agencies in achieving technological self-sufficiency in the area of symbiotic nitrogen fixation. The goal of all NifTAL programs is to increase agricultural productivity and, by promoting legume cropping systems, to improve soil fertility and reduce dependence on costly nitrogenous fertilizers.

ANNUAL RESEARCH REPORT

GENERAL BACKGROUND

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

NifTAL's strategy is to:

- Identify effective strains of *Rhizobium*;
- Demonstrate the benefits of *Rhizobium* inoculants by field trials throughout the tropics;
- Provide technical assistance to institutions trying to maximize biological nitrogen fixation by legumes through training of key personnel in *Rhizobium* technology, through provision of germplasm inoculum or information resources, or through expert consultancies in inoculant production.

CONTRACTED OBJECTIVES

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

- A collection of *Rhizobium* strains, including strains which have been tested and proven on targeted legumes, for distribution to researchers worldwide;
- Improved inoculum delivery techniques to ensure effective, dependable field inoculation;
- Improved techniques and cultural systems to exploit more fully the nitrogen contribution of legumes in tropical cropping systems;
- Training programs on applied research and technology of *Rhizobium* bacteriology for technicians and scientists in tropical countries; and
- A formal network of cooperators in the tropics for field testing of *Rhizobium* inoculants.

ANNUAL ACCOMPLISHMENTS

Inoculation Methodology

A dilution technique for the production of legume inoculants with gamma-irradiated peat:

The effects of four diluents of different nutrient status (deionized water, yeast-mannitol broth, yeast-water and yeast-sucrose broth) on the subsequent growth and survival of rhizobia in peat under long-term storage conditions were evaluated.

Two distinct strains of rhizobia, a fast-growing *R. phaseoli* (TAL 182) and a slow-growing *R. japonicum* (TAL 102) were cultured in gamma-irradiated peat from two different sources. Inoculants with each *Rhizobium* strain were prepared using the four diluents and tested for changes in effectiveness duo to strain-diluent interaction.

Data were subjected to a three-way analysis of variance (p=0.01) to determine the influence of the initial inoculum level on subsequent growth and survival of *R. phaseoli* (Appendix 1, Table 1, page 40) and *R. japonicum* (Table 2, page 40), and significant differences were obtained. Differences were due to the initial low titre of the injected (diluted) broth and the high population attained after one week of incubation. No significant changes in the population were detected after the first week.

Maximum population attained in the peat was independent of the initial numbers of rhizobia present in the broth inoculum for both strains. This suggests that small volumes of high titre (e.g. a x 10^9 cells/ml) broth cultures of *Rhizobium* may be diluted 10x, 100x, or 1000x prior to injection into gamma-irradiated peat. The maximum population will still be attained in one or two weeks when the peat is incubated at 28° C.

The four diluents did not differ significantly in their ability to support growth and survival of rhizobia (Appendix 1, Tables 3 & 4, page 41). There was gradual decline in the population with time; however, this is an occurance that is usually observed during long-term storage at high temperatures. There were no changes in strain effectiveness as affected by the different diluents (Table 5, page 42). All plants grew normally and formed effective nodules with red/pink interiors.

Inoculant production:

A small-scale, peat-based inoculant production plant was put into operation. Peat is packaged in 50g quantities in polyethylene bags (1.5 mm thickness) and irradiated at 5 x 10^6 rads. Liquid broth inoculum, for injection into the peat, is grown using the shaker method.

Strains selected for inoculant production are from NifTAL's germplasm collection. These strains were tested extensively in the laboratory, greenhouse, and field. Inoculant for a particular legume species consists of a mixture of the three best strains. Liquid cultures of these strains are grown separately and mixed prior to injection into the peat.

Mixed inoculants for the 12 legumes selected for testing in the International Network of Legume Inoculation Trials are produced in this plant and sent to Cooperators. To facilitate strain identification in nodules by serological methods, the three *Rhizobium* strains for each legume species have been tested for absence of cross-reactions. The various strains and their respective hosts for the Network are listed in Appendix 1, Table 6.

This inoculant plant is also used as a tool for teaching participants in the NifTAL intern training programs some of the practical aspects of inoculant production. Additionally, it serves as a model inoculant production plant using appropriate technology for the developing countries.

Antiserum bank:

Antisera are being developed for each of the strains recommended to Network Cooperators. Since the inoculant for each legume species for the Network is a mixture of 3 antigenically-distinct strains, antisera need to be developed for each of the component strains of the mixture. Antisera for 36 different strains of *Rhizobium* for the 12 Network legumes are in production. Antisera for strain identification by agglutination, fluorescent antibodies, and immunodiffusion have been prepared. These are lyophilized in small vials, and sealed under vacuum to insure stability during long-term storage. These antisera provide a quick, reliable method for identifying specific *Rhizobium* strains.

To date, an average of 120 mls of antisera per strain have been prepared against 18 of the 36 Network strains for agglutination. An average quantity of 40 mls of antisera for immunodiffusion has been prepared against four strains, and an average of 70 mls of fluorescent antibody has been produced against five strains. Antisera possess a minimum agglutination titre of 1:2560 and fluorescent antibody preparations have a minimum fluorescent titre of 1:10.

The antiserum bank provides antisera to Network Cooperators, as well as to the NifTAL scientific staff.

Identification of Tolerant Strains

In identification of rhizobia with tolerance to soil chemical stresses, cases are first identified in which selection of rhizobia is justified. Valid methods of screening are then developed. Chemical stresses under study include soil acidity, salinity, and phosphate deficiency.

Soil acidity factors:

Previous research has shown that high aluminum, as well as low pH, was likely to limit rhizobial growth in acid soils; that strains differed in sensitivity; that sensitivity could be detected by growth tests in appropriate laboratory media; and that sensitivity in laboratory tests was a useful predictor of failure to nodulate in acid soil. No indication of the manner in which acid/Al stress limited growth, or whether tolerance was a stable, uniform property of bacteria of a given strain was available. Partial answers to these questions have been found in work completed this year at University of California, Davis. (See Figures 2-3, pages 44-45 and Table 7, page 46.)

In synchronous culture, the main effect of acid/Al was to slow, and then disrupt, cell division without mortality of nondividing cells. No sign of enrichment of tolerance resulted from successive subculture in liquid medium under stress (72 generations), or from isolation of the odd colonies that several strains were able to produce on a specially designed acid/Al-saturated agar medium. It was concluded that Al toxicity primarily impairs cell division (as in plants); and that a strain's tolerance is a stable property, unlikely to be accidentally lost in culture or to be easily improved without mutagenesis.

Screening for stress-tolerance requires a correct choice of degree-of-stress to apply. This is not helped by ignorance about conditions in the legume rhizosphere. The possibility that legume seedlings significantly acidify their rhizosphere has been tested with cowpea and soybean by direct measurement with glass microelectrodes. The results indicate that significant acidification requires almost absolute absence of nitrate; in fact, with reasonable levels of soil nitrate, the host legume might be induced to raise the rhizosphere pH enough to assist rhizobial colonization.

Two cases have been detected where selecting rhizobia for acid tolerance is not justified. All of 13 currently-available strains of soybean rhizobia effectively nodulated 9 varieties of soybean even in soils too acid (pH 4.4-4.5) for the host to grow. And 55 cultivars of cowpea in soil at pH 4.4 in large containers in the open were all nodulated fully effectively by the tolerant rhizobia TAL 169, TAL 174, and TAL 309 although growth of many of the host cultivars was inhibited severely by Al toxicity. In both cases, selection priority is due the host; not the rhizobia.

Salinity:

Earlier research had shown that chickpea (*Cicer arietinum*), though commonly grown in salt-affected soils, is salt-sensitive, especially when symbiotic with *Rhizobium*, and that the chickpea rhizobia can themselves tolerate several-fold higher salt concentrations than the plant. Attempts to select rhizobia capable of good symbiotic performance in saline media had little success. Clear evidence is now available that the main difficulty is nodule function. And 35 cultivars of chickpea have been screened in search of types that might be more tolerant than the large-seeded White Spanish landrace.

Phosphate deficiency:

Phosphate deficiency may be the most widely distributed factor limiting symbiotic nitrogen fixation after drought and lack of a legume. Phosphate nutrition of legumes has received much attention by researchers, but P nutrition of *Rhizobium* has been ignored. A series of tests with soybean rhizobia has shown that:

- Rhizobia can store large amounts of P from high-P media, *e.g.* artificial media, nodules, and fertile soils (P in solution at 10^{-5} to 10^{-3}M).
- Stored P can support up to 5 generations of growth without external P supply. This characteristic varies between strains. It may be useful to arrange for high stored P in inoculants.
- It is possible to maintain P in liquid media at concentrations ranging between 3×10^{-8} and 3×10^{-5}M--the important range in

soils and rhizospheres and in cultures where cell density is low (10^6/ml) and P in solution is buffered by desorption and dialysis from reserves of P sorbed on iron oxide.

Using the above system, it can be seen that strains differ in ability to grow at realistically low concentrations of P. Some strains (USDA 110 and CB 756=TAL 309) do well at 3×10^{-8}M, and are unlikely ever to be P-limited in the field. Others are unable to grow normally even at 10^{-5}M, and should be P-limited even in normal soils.

Very tentative evidence from plant growth tests in P-deficient sand suggest that the above laboratory tests might agree with symbiotic tests in P-deficient soil. (See Table 8, page 46.)

Currently, procedures are being refined and NifTAL's Network Collection as well as a representation of nontropical fast growers are being screened. (See list of relevant publications, Appendix 5, page 87.)

Legume Inoculation Trials

International Bean Inoculation Trial:

Ten strains of *Rhizobium* isolated from *Phaseolus vulgaris* were supplied by CIAT and 2 strains were supplied by NifTAL in an inoculation trial at Kuiaha, Maui. The experimental design used was CIAT's IBIT (International Bean Inoculation Trial). Controls of no-N, uninoculated and plus-N, uninoculated (-N, -R, +N-R) were included. The treatments were replicated 4 times. This trial is in the field during the summer of 1980. Viable cell counts on peat inoculum and on inoculated seeds were performed at the time of planting. First harvest (at 25 days) was scheduled for July 7th.

International Network of Legume Inoculation Trials/Experiment A Hawaii trials:

To determine what problems might be encountered in applying a standardized inoculation trial throughout the tropics, "Experiment A" was installed at the Kuiaha Experiment Station, Maui. It was a three-replication trial with 'Bountiful', a locally-used string bean (*Phaseolus vulgaris*) with good resistance to mildew. The maximum-fertility treatment received 1000 kg/ha of phosphorus and 8500 kg/ha of dolomite to raise the pH to 6, plus standard amounts of other fertilizers. The farm-fertility treatment was limed to pH 5.2 and received 100 kg/ha P. Planting date was May 4, 1979, and string beans were harvested on July 2, 1979. Plots were sampled on June 5 for acetylene reduction tests, dry matter production, and chlorophyll content of leaves.

Data from the final harvest, acetylene reduction, and leaf chlorophyll content are given in Table 9, pg. 47. Leaf chlorophyll content showed that inoculated or nitrogen-fertilized plants showed a significantly higher chlorophyll content than uninoculated plants given no nitrogen fertilizer. Acetylene reduction data show a significant response to the maximum fertility level and also a significant benefit from inoculation. Yield was significantly increased by the maximum-fertility treatment. In the farm-fertility treatment nitrogen-fertilized plots showed significantly greater yields than inoculated plots or uninoculated controls. In the maximum-fertility treatment the nitrogen fertilized plots were not significantly different from the uninoculated control or the inoculated plots.

A second experiment was planted with soybeans (*Glycine max*) using the same treatments on the same plots as in the first experiment. No additional fertilizer was used, except that nitrogen was reapplied to the N-treatment plots. The data for yield, chlorophyll content of beans, and acetylene reduction are shown in Table 10, pg.48. The chlorophyll content again was significantly less in the uninoculated control plots compared to the nitrogen fertilized or the inoculated treatments. Acetylene reduction data indicate a lack of soybean rhizobia in this soil with all inoculated plots showing minimal activity while inoculated treatments show a good response. Yield data show a good response to the maximum-fertility treatment. The effect of the fertility level on the inoculation treatment was that yields were significantly higher in the maximum-fertility level plots than in the farm-fertility level plots. A similar but not significant effect was observed in the first experiment. Results in the second experiment were quite variable due to an outbreak of fusarium wilt which raised the coefficient of variation to 35% compared to 16% in the earlier experiment.

One effect of the maximum-fertility treatment was the stimulation of the mineralization of soil nitrogen by raising the pH and providing more available phosphorus. This effect was previously observed at this site in a pH-gradient study. The increase in yield due to nitrogen fertilizer was about the same for the farm and the maximum-fertility levels; however, the maximum-fertility treatment raised the yield of the inoculated treatment while the farm-fertility treatment resulted in no response to inoculation, indicating that fertility level controls the nitrogen fixation capability of the soybeans.

An INLIT (Experiment A Design) was also completed with chickpea (*Cicer arietinum*) at the Kuiaha, Maui, site. Preliminary data indicate that inoculation increased vegetative yields significantly over uninoculated, zero-N controls. (See Table 11, pg. 49 for mid-flowering data.)

Two other experiments were started but had to be abandoned. An experiment at Kuiaha was planted with leucaena (*Leucaena leucocephala*) in December 1979. This trial was abandoned after unusually cool, very wet weather resulted in slow growth of leucaena and rapid growth of winter weeds. Close spacing of rows made weeding very difficult. Frequent rains also prevented field work for many consecutive days. A mung bean (*Vigna radiata*) trial planted at the Hamakuapoko site was also abandoned. A severe attack of mildew resulted in death of many plants and an inadequate stand.

These field trials have demonstrated the hazards of field trials. There is a need for more than 3 replications to reduce the change of a high coeffecient of variation when disease, pests, or climate factors arise. Crop protection measures are being integrated into planting procedures for further trials in an attempt to determine their effectiveness. It is obvious, however, that not all field trials can be expected to produce usable data.

NO_3^- effect on nodulation in soybeans:

A prime advantage of legume use is the ability of the symbiotic plant to fix nitrogen from the atmosphere. Preliminary nodulation and continued N-fixation, is reduced by external NO_3^- in soybeans (See pg. 50.) However, it is not known exactly how the reduction is effected. By working with both *Rhizobium* and plant, it may be possible to produce a symbiosis which is much less affected by NO_3^-. This system would be of particular advantage to developing countries in that it would improve the efficiency of mixed cropping systems where the cereal component must make use of soil or fertilizer NO_3^-.

Initial evaluation of 25 *Rhizobium* strains on soybeans grown in pouches suggested differential effects of NO_3^- on nodulation; however, where the extreme cases were tested in pot experiments there were no significant nor apparent differences (Figure 4, pg.50.) Nodules could be produced in solution culture containing NO_3^- when a sufficiently high level of inoculant was applied. Three generations of isolations are therefore made using this technique and the resultant strains are being compared against their parents for ability to nodulate and grow in the presence of NO_3^- in a replicated pot experiment.

Seed for 426 soybean cultivars is being tested for nodulation and nitrogen fixation in the presence of NO_3^- in a four times replicated field experiment. The goal is development of a complete symbiotic system for reduction of NO_3^- inhibition. To increase the likelihood of success, cultivars were obtained from several locations around the world and include both improved and original varieties. These experiments are in the field during the summer months of 1980.

The effect of nitrate concentration on nodulation and nitrogen fixation:

The inhibition of nodulation by nitrate has been observed by scientists for many years, as have the benefits of "starter" nitrogen in increasing yield and nitrogen fixation. To study the effects of nitrate nitrogen on nodulation, nitrogen fixation, and growth of soybean *(Glycine max)*, a solution culture approach was used. Using this method the concentration of the nitrate ion can be easily measured and uniformly maintained throughout the pot.

The effect of nitrate concentration was studied using treatments consisting of 0, 0.5, 1.0, and 2.0 mM concentrations of nitrate in a modified Hoaglund solution, inoculated with TAL 379 (10^9 rhizobia per liter). A fifth treatment was maintained at 0.5 mM nitrate concentration for two weeks after emergence; then replaced with a nitrate-free solution until harvest eight days later, to observe the recovery of nodulation and acetylene reduction of soybean plants.

The results given in Table 12 (page 51) show that although pots were inoculated with TAL 379, an effective soybean strain, nitrogen severely reduced the number of nodules. Nodule number ranged from 90 for the no-nitrogen treatment to 3 for the high-nitrogen treatments. When nitrate was removed from the solution in treatment 0.5/0, nodule mass and number increased very rapidly, demonstrating the starter nitrogen effect.

The 0.5 M NO_3 treatment had 12 nodules per plant, but these did not show up until the last week. Nodulation may have been affected by depletion of nitrate in the nutrient solution. Although nitrate was renewed twice daily, the supply of nitrate in this treatment was depleted several times for short periods after the plants became large and their demand was great.

The results (Table 12) also show a very large increase in dry matter of the tops and roots due to nitrogen. Dry matter production of tops and roots was nearly five times as great in the nitrate treatments as in the control treatment. The no-nitrogen treatments were well-nodulated, but the plants had received no additional nitrogen until eight to ten days after emergence when nodules were visible. This delay prevented the plants from attaining the dry matter yields obtained from nitrate-fertilized treatments. The 0.5 mM-NO_3 treatment had significantly less root growth than the 1.0 and 2.0 mM-NO_3 treatments, but this was probably due to the exhaustion of the nitrate during the period of high demand. The 0.5/0 treatment shows a substantial reduction in top growth, but a significant increase in root mass, compared to the other nitrate treatments. The change in nitrogen status resulted in increased root growth and nodule development at the expense of top growth and a substantial reduction in total dry matter.

Acetylene reduction values were also higher in the nitrate treatments than in the no-nitrogen treatment. The larger plant produces more photosynthate. Due to lack of N this photosynthate cannot be used in top growth, but it is used in production of greater nodule mass. This increases the capacity for biological N fixation and results in higher acetylene reduction values.

It is frequently noted in the field that legumes grown in soils which supply nitrate to plants are frequently well-nodulated, while low levels of nitrate in well-mixed solution culture suppress nodulation almost completely. This is thought to be due to the lack of mixing in soils resulting in uneven distribution of N and a very low concentration of N at the root surface. To test this hypothesis, a comparison of growth, nodulation, and acetylene reduction was made under growth conditions employing different degrees of mixing.

Soybeans were grown with and without N in solution culture employing three different cultural methods. The methods were:

- Solution culture continuously aerated and mixed with a stream of air bubbled through a porous stone;
- Fine gravel media with continuous circulation of solution from a reservoir at a rate of about 15 ml/min; and
- Sand culture in which the solution was changed daily.

In the first two treatments solutions were analyzed daily and the concentration of nitrate was maintained at 1 mM. In treatment 3 the solution was changed daily, but when plants became large they depleted the nitrate supply between renewals.

The results in Table 13 (page 52) show that, when the plants were grown without nitrate, the cultural method had no significant effect on yield of tops and roots, nodule mass, or acetylene reduction. However, when the plants were grown with nitrate, there was a large and significant effect of cultural method on yield of tops and roots, nodule mass, and acetylene reduction. There was also a marked difference in yield of roots and tops between the pots receiving nitrate nitrogen and the ones dependent on biological nitrogen. As the degree of mixing decreases from solution to gravel to sand, plant growth decreases and nitrogen fixation decreases.

In a subsequent experiment, the effect of nitrate present in the solution surrounding a root on nodulation was studied in a split root experiment. A single lateral root was used so that the test would have a minimal effect on the plant as a whole. All solutions were inoculated with 10^9 rhizobia per liter. Treatments were replicated 5 times. The treatments and their effect on the number of nodules per single root are given in Table 14 (page 53).

In a separate trial, six additional plants of treatment 2 were grown and none of the single roots had any nodules. Out of eleven replicates of treatment 2, only one of the single roots nodulated. The main roots of the two treatments which received no NO_3 on the main root were well nodulated. and had over 100 nodules per plant on the average. The treatment receiving nitrate on the main root (treatment 2) had no nodules on any roots of the plants.

These experiments indicate that nodulation is controlled by the effect of nitrates on the entire plant, and not by the presence of nitrate outside individual roots or by nitrate being absorbed by such roots. Further work needs to be done on this problem since the number of replications in this experiment was small, and since only one host and one strain was studied.

Chickpea nodulation:

A field experiment in conjunction with Dr. T. S. Sandhu of the Punjab Agricultural University employed a 10 x 4 cultivar-by-strain evaluation with chickpea but was abandoned due to severe wilt. It did serve the useful purpose, however, of screening the readily-available varieties of chickpea for growth under Hawaiian conditions. Results indicated that L550, G130, and possibly the commercial Burpee varieties were best suited. This work with chickpea will be continued and extended in a breeding program in India with T. S. Sandhu and P. J. Dart of ICRISAT. The goal is increased N fixation in chickpea.

Allelopathy

Six species of *Rhizobium* were grown in yeast-mannitol broth media containing five concentrations of each of five phenolic compounds. Optical density readings of broth cultures were taken every other day for twelve days. The treatments were replicated three times.

Viable cell counts were taken of phenolic controls at 0-time and at each optical-density reading to establish growth curves. Optical density readings indicated that all phenolic acids completely inhibited all species of *Rhizobium* tested at 5×10^{-3}M concentration. Phenolic x *Rhizobium* species interaction was apparent in various degrees of inhibition of rhizobial growth at 10^{-3}M and 10^{-4}M concentration. Very little inhibition was evident at 10^{-5}M and 10^{-6}M concentration. (See Figures 5 & 6, pages 54-55).

A third experiment employed the *Rhizobium* spp. from the first experiment plus TAL 309 from the second and phenolic acids at 5×10^{-3}M concentrations. Phenolic acids were pH-neutralized with KOH prior to adding to YM media. Controls included 0-concentration phenolics with plus- and minus-acidity factors, and phenolics which had not been neutralized. Optical density and viable cell counts were conducted as in parts I and II.

All phenolic acids at 5×10^{-3}M concentration inhibited all strains of *Rhizobium* when the phenolic acid was not neutralized. *Rhizobium* strain x phenolic interaction was apparent in various degrees of inhibition of *Rhizobium* growth when the acidity of the phenolic was neutralized by KOH. (See Figure 7, page 56.)

Agroforestry

Seeds of legumes for agroforestry systems are being requested from those who are able to supply them. A seed accession system has been started in conjunction with Dr. J. Brewbaker.

A nursery stock of *Acacia auriculiformis* has been started in NifTAL greenhouse. The trees are intended for mass planting on NifTAL site for comparisons with *Leucaena leucocephala*, 'K-8'.

Training in Rhizobium *Technology*

To meet the need for scientists and technicians with the skills for competent, production-orientied research and technological development in tropical countries, NifTAL offers three training programs.

Graduate Research Program:

The Graduate Research Program is an ongoing part of the Project. MS and PhD candidates who meet University of Hawaii entrance requirements and who are interested in research projects in the legume/*Rhizobium* symbiosis can be awarded research assistantships. Emphasis is on third-world students or students with a commitment to work in development. Four students (two MS graduates and two PhD graduates) had completed graduate programs previous to FY 1979-80. Two more graduates completed degrees during the year. (See page 65 for listing of NifTAL-supported graduate researchers.)

Intern Training Program:

The Intern Training Program offers the opportunity for key personnel from legume research programs to acquire the techniques of *Rhizobium* technology in a hands-on, informal course of study at NifTAL's Hawaii Labs. Interns participate in lab, field and greenhouse research with scientific and technical staff in addition to receiving individually-tailored instruction in legume microbiology and agronomy. Training covers all practical skills needed to conduct appropriate back-up technology to aid in national programs aimed at better utilization of legumes. The duration of training is 2-4 months, depending on the trainee's background. Trainees who successfully complete the program are awarded a Certificate of Achievement. (See list of intern trainees, page 65).

Regional courses:

NifTAL's training staff have developed a six-week intensive course and a training manual, *Practical Exercises in Legume*/Rhizobium *Technology* to complement the instruction. Regional courses that can accomodate 15 participants are mounted in conjunction with host institutions in tropical countries. About half the participants are selected from the host country and half from neighboring countries in the region. The course is practical and emphasizes not only the microbiological skills for handling rhizobia, but all those laboratory, greenhouse and field procedures for testing, producing and using rhizobia in legume cropping systems.

In August-September 1979 NifTAL and the Nairobi MIRCEN (Microbiological Resources Center) sponsored a Regional Course at Nairobi, Kenya. Twelve trainees, selected by the University of Nairobi, attended the session. Two members of NifTAL's training staff went to Nairobi, prepatory to the course, and arranged for course materials to be built or purchased. They were also responsible for the bulk of the instruction with guest lectures contributed by other US and Kenya experts. At the completion of the course a questionnaire was distributed to determine trainee response. (See page 65 for a list of participants, page 67 for sample questionnaire with compiled results.)

Courses are planned for SE Asia at Malaysia (co-sponsor, MARDI) in November 1980 and for Central America and the Caribbean in Mexico (co-sponsor, Colegio de Postgraduados) in June-July 1981. Preparations for these courses are in progress.

Courses sponsored by other institutions:

NifTAL has committed its resources to support tropical institutions involved in training. The MIRCEN at Porto Alegre, Brazil sponsored a short course in *Rhizobium* technology in June-July 1979. NifTAL supplied an expert instructor to this course. For the Porto Alegre course in the summer of 1980 NifTAL increased its support. Seven trainees were sponsored by NifTAL, and an instructor and copies of NifTAL's training manual were also supplied. This level of support is planned for additional courses in FY 1980-81.

Training manual:

The NifTAL training manual, *Practical Exercises in Legume/*Rhizobium *Technology,* is highly regarded by institutions involved in BNF training. This manual was first made available in FY 1979-80. Revisions incorporating comments of students and instructors will be made before the Malaysia course. A Spanish language translation has also been prepared and is in the final editing stage. That version will be available for the Mexico course.

Audio-tutorial modules:

To strengthen the training services provided by the NifTAL Project, the College of Tropical Agriculture and Human Resources and the NifTAL staff have explored the possibility of developing self-instructional programs for trainees. A two-day workshop hosted by NifTAL on Maui and presented by CTAHR's Curriculum Specialist, Dr. Frank Hiob, gave staff members basic techniques in course design and module development.

Preliminary plans are to assemble slide/tape modules for teaching exercises to intern trainees in Hawaii. It will then be possible to evaluate the modules at overseas locations. Through the technique of successive approximations the modules will be revised until they meet training objectives set out by the Training Section. Dr. Peter Graham of CIAT has agreed to participate in a final review capacity.

The Department of Agronomy and Soil Science has provided photography lights and audiovisual carrels. Arrangements have been made with Maui Community College to provide equipment for producing tapes.

Network Establishment

Participants in the International Network of Legume Inoculation Trials, which was initiated in 1979 by scientists and administrators from many national and international research institutions, have recognized that there is a real need to determine whether inoculating tropical legumes with their appropriate companion rhizobia will increase the crop yield without dependence on N fertilizers. This can only be accomplished by experimentation under realistic field conditions and in the numerous different tropical environments in which legumes might be grown. Past field trials, while producing some encouraging results, have seldom included an assessment of the quality of the inoculant at the time of use, and the trials have not been coordinated by use of a standardized experimental design. Meaningful interpretation of the trials has, therefore, been difficult.

NifTAL was assigned major promotional responsibility for identifying Cooperators and coordinating the standardized inoculation trials throughout the tropics. Cooperators are receiving support services to conduct the trials properly. Arrangements will be made for production and supply of inoculant in the Cooperator's country. Key personnel from tropical regions are receiving training in legume/*Rhizobium* technology, through NifTAL's training programs.

Design of the experiments:

In the first round of field experiments, a general inoculation recommendation with strains presumed to be the best available will be made. A negative result in these experiments is not definitive evidence that the legume in question will not respond to inoculation, since the strains of rhizobia were not selected specifically for the legume variety of interest to the individual Cooperator nor for the Cooperators' local soil and climactic conditions.

For this reason there are two additional steps in the three-stage experimental program. Experiments in the second stage are designed to compare strains, so as to select the best strain for the legume variety under prevailing soil and climatic conditions. The Cooperator will then be able to perform a definitive legume inoculation experiment with a strain of *Rhizobium* (or a mixture of strains) specifically selected for the local variety under local conditions. The opportunity for a positive yield response will, therefore, have been maximized.

Cooperators are encouraged to undertake trials with soybean *(Glycine max)* and leucaena *(Leucaena leucocephala)* to permit sufficient between-site comparisons. These legumes have specific *Rhizobium* requirements and may respond well to inoculation.

Other legumes which are likely "targets" are peanut *(Arachis hypogaea)*, chickpea *(Cicer arietinum)*, lentil *(Lens culinaris)*, common bean *(Phaseolus vulgaris)*, cowpea *(Vigna unguiculata)*, and the forages *Centrosema pubescens*, *Desmodium intortum*, and *Stylosanthes guianensis*.

The infectiveness and persistence of introduced strains is being monitored by specific antisera developed by NifTAL.

Progress in the network trials:

For the past year, NifTAL has been contacting scientists and researchers throughout the tropics, and the response from these potential Cooperators has been very positive. Informal commitments have been made for 36 trials in 17 countries and many other potential trials are in the "follow-up" stage. Regional Coordinators have already visited many of the Cooperators in SE Asia, South America, and Africa.

The Americas Network:

The Americas Network includes South and Central America, the Caribbean, Mexico, and certain areas of the United States, including Hawaii. Arrangements for trials have been made through on-site visits and mail communications. To date, 58 trials have been or will be sowed in the Americas. (See Appendix 4.)

In promoting the Network, special attention has been paid to insure that the same strains are used across all sites and that serological identification of nodule rhizobia are made. Soils, climates, and cultural practices vary greatly.

At most locations the facilities are available to perform the microbiological aspects of the trials. Where necessary, training or support through a properly equipped institute in the region has been arranged. Short term on-site training has also been requested.

At many sites participation in the Network has not been possible due to a lack of potential cooperator funds or personnel to carry out the trials. In some cases, material support has been provided in the form of fertilizers or seeds, but we have been prevented by our own budget from lending more financial support where it would have been beneficial. Since Spanish is the predominant language in the area, the experimental procedures have been translated to Spanish.

Strong support for the Network has been evidenced by such international research programs as INTSOY, NCSU Tropical Soils Project, Microbiological Resource Centers (UNEP/UNESCO) and the Benchmark Soils Project. Substantial interest in NifTAL's activities has also been expressed by the USAID country missions and logistical support has been provided in several cases. Efforts are being made to strengthen these contacts and forge new linkages with other organizations working in the less developed countries of the tropics.

The Africa/Mid-East Network:

The Africa/Mid-East Network includes all of Africa plus Turkey and Syria. Site visits have been made in Tanzania, Malawi, Zambia, Kenya, Sudan, and Egypt after earlier communications by mail. In September 1981, West African Cooperators identified through IITA in Nigeria in discussions with Dr. Ed Pulver (IITA Microbiologist) during his NifTAL visit will be contacted for site visits.

The Network and the Experimental Design were very well received by African Cooperators as it was a first exposure to field inoculation trials. All potential Cooperators expressed keen interest in setting up experiments at more than one site. In Sudan and Egypt, where horse bean *(Vicia faba)* is an important grain legume, Cooperators expressed a desire to include it on the list of key legumes.

Most of the experiments will be planted in the 1981 season as many of the Cooperators are already involved in their local research programs for

1980. Cooperators were encouraged to incorporate the Network Experiments into their local research programs planned for 1981. In Zambia, Kenya, Rwanda, and in Egypt, Cooperators have indicated they will try to have trials in the ground by the end of 1980. In other locations, however, no experiments are expected until definite commitments are made on financial support to the Cooperators.

The timely arrival of inoculants for the experiments could be a serious problem in some of the African countries. Cooperators in Zambia and in Egypt were concerned about delays in the local postal systems. To facilitate timely arrival of inoculants USAID missions in various countries are being contacted for permission and assistance for cooperators to receive the inoculants through the AID missions.

The Asia Network:

Cooperators in India, Sri Lanka, Pakistan, Bangladesh, Malaysia, Thailand, Indonesia, and the Philippines have received site visits. Initial interest in the Network was good, both among individual researchers and with administrators of research institutions and government agricultural officials.

A total of 78 experiments have been planned for the calendar year 1980. Some trials have already been planted. The bulk will be planted in the season now in progress in South Asia or the planting season which extends in various parts of the region from September through December 1980.

The Asia Network consists of three separate types of cooperative efforts. Cooperation with individual research institutions or scientists, cooperation with institutions that have many sites within the country, cooperation with cooperatives in country Networks, and combinations of the above.

The most effective way to operate in this region is to utilize, where available, in-country or regional networks. It is impossible for the Regional Coordinators to visit individual sites as frequently as would be desired. Also, international mail is often slow. Where such in-country or regional networks are available it is possible for an In-country Coordinator to perform within the country; thus giving the NifTAL Regional Coordinator one point of contact for several experiments.

Negotiations are in various stages of progress with the:

- Coordinated Research Program for Pulses - India
- Coordinated Project for Dryland Agriculture - India
- Indian Council of Agricultural Research - India
- Indo-US Joint Scientific Commission - India
- Director of Agriculture, UP State - India
- Pakistan Agricultural Research Council - Pakistan
- Coordinated Soybean Scheme - Sri Lanka
- Bangladesh Council of Agricultural Research - Bangladesh
- Malaysian Agric. Research and Development Inst. - Malaysia
- Scientific Council - Malaysia
- Philippine Council for Agric. & Resources Research - Philippines
- Crops Research Institute - Indonesia
- Soils Research Institute - Indonesia

(See Appendix 4 for statistical breakdown for Asia.)

DISSEMINATION AND UTILIZATION OF RESULTS

Distribution of Rhizobium *cultures by the NifTAL Project; July 1, 1979 - June 30, 1980*

	Country	Number of Requests
	Malaysia	1
	Spain	2
	Thailand	3
	U.S.A.	15
	India	2
	Brazil	1
	Canada	1
	Argentina	3
	Kenya	2
	Venezuela	1
	Germany	1
	Sri Lanka	2
	Indonesia	1
	Nigeria	1
	Mexico	1
	Dominican Republic	1
TOTAL:	16 countries	38 requests

Total: 354 cultures distributed

On Beads - 200
On Peat - 5
On BTB Slants - 149

Information Transfer

Scope of Information Section:

The scope of NifTAL's information dissemination activities was broadened in FY 1979/80. The newly-formed Information Section is staffed by an Information Specialist, a Bibliographer (previously on staff), a part-time Artist, and student assistants with responsibility for editorial, circulation, and clerical chores. The activities of the Information Section include writing, editing, compiling, collecting, designing, illustrating, producing, and distributing the information outputs of the Project.

An Information Office was set up at Manoa, and the Information Specialist position is a split appointment between the Oahu and Maui offices. This enables better service in production of publications and provides a liaison point for Maui staff when they are on Oahu.

Support publications - Network and training:

A number of publications were generated in conjunction with the Network and NifTAL's training activities. Support publications covered a wide range of types and topics.

The Proceedings of the 1979 Planning Workshop were produced and distributed to all Workshop participants and potential Cooperators in the Network. Two brochures, one describing NifTAL and one explaining the International Network of Legume Inoculation Trials, were distributed throughout the tropics. The document giving the experimental design for the trials was also produced, and this document continues to be refined to simplify Cooperator inputs.

Since NifTAL training is available to potential Network Cooperators and other interested parties, two brochures describing NifTAL's training opportunities were designed, produced, and distributed. A manual, *Practical Exercises in* Rhizobium *Technology*, has been produced for use in NifTAL training, and materials for the manual continue to be updated and simplified. This learning tool is in demand by other institutions involved in training, and a Spanish language version is now in production. Course announcements are another output associated with the training function. "Certificates of Achievement" were designed to award trainees who complete NifTAL's programs. Finally, a newsletter, "Notes from NifTAL," is produced twice yearly and distributed to all past and present trainees.

Audio-tutorial module workshop:

In conjunction with training activities, an audio-tutorial module is in the planning stage. A two-day workshop for staff members involved in this activity was sponsored by the Information Section. Dr. Frank Hiob, Curriculum Specialist and Acting Assoc. Dean of the College of Tropical Agriculture and Human Resources led the workshop participants through the philosophy of audio-visual instruction, education needs assessment, and steps in course design.

Document collection:

NifTAL headquarters is separated by ninety miles of ocean from the University of Hawaii library. NifTAL's bibliographer serves as an information link with that library and with the scientific community beyond. An extensive but highly specialized document collection, along with a core of basic reference books, serve the staff and trainees as a library for special research purposes. These resources are also available to eligible clients abroad.

During the period July 1, 1979 - June 21, 1980, the following activities were recorded:

Documents added to collection:
- 464 scientific articles
- 12 books
- 44 newsletters, reports, etc.
- 57 journal issues

Documents sent out on request:
- 264

Number of countries involved in information exchange:
- 88

As they are acquired, all documents are placed on display for at least one week as a current awareness service to staff and trainees. The weekly printout of the Automated Subject Citation Alert (ASCA) purchased from the Institute for Scientific Information (ISI) is also posted on a current awareness bulletin board, as are meeting announcements and other items of interest. (A separate posting of official announcements and university information is maintained at the mail distribution point.) As they travel, scientific staff are able to pass this current information on to Cooperators and other colleagues.

The 1980 NifTAL brochure carries the information that photocopies of specific documents are available to Network Cooperators, former NifTAL trainees, and to researchers working in countries eligible for USAID support. The promulgation of this information, along with the growth in the number of NifTAL clients resulting from Network expansion and new training courses, have already resulted in a considerable increase in document requests. Travel reports by network coordinators indicated considerable enthusiasm for this service.

Bibliographic services:

A bibliographic update to the 1978 publication "The Legume/*Rhizobium* Symbiosis in Tropical Agriculture" was completed in FY 1979/80. This update contains 697 annotated citations pertinent to the legume/*Rhizobium* symbiosis. Copies of all documents listed are held at NifTAL.

The mechanism for providing bibliographic listings on specific topics, a service mentioned in the NifTAL brochure, is operational. This will greatly improve the current awareness service to the NifTAL staff and to outside clients. It offers the opportunity to provide, within the rather narrow limits of our interests in the legume/*Rhizobium* symbiosis and legumes in cropping systems, information service equivalent to that of a full research library.

Agroforestry questionnaire:

More than 200 questionnaires were sent to various scientists in 50 countries to survey sources of seed for 36 species with N-fixing potential to be appraised for agroforestry systems. The questionnaire also solicited comments on growth, habit, ecology of species, references, and suggestions for additional species for agroforestry appraisal. (See Appendix 2.)

Approximately 60 questionnaires have since been returned, many with pertinent publications included. The comments, references, etc. are being compiled.

BNF publications:

The BNF research teams at the four institutions which are members of the Consortium for Biological Nitrogen Fixation in the Tropics (Cornell, NCSU, Hawaii, and Puerto Rico) and NifTAL's scientific staff have been strongly aware of the desirability of improved communication in the broad field of BNF research. The contract directors of the five projects involved agreed to joint sponsorship of the *BNF Bulletin.* This six-page, two-color newsletter contains articles on research activities, personnel news, and other informative materials directed at technical and non-technical audiences. The newsletter is written, produced, and distributed by NifTAL's Information Section.

News releases and displays:

A number of news releases describing NifTAL's work and its relationship to the non-scientific community were prepared by the Information Section. These were used by the following news media: *Maui News, Honolulu Advertiser, Star-Bulletin, Malamalama,* and *News You Can Use.*

A three-dimensional display on NifTAL activities was produced for the reception lobby of the administrative area of the College of Tropical Agriculture and Human Resources. Materials describing the benefits of biological nitrogen fixation were produced and supplied to the Department of Agronomy and Soil Science for the Hawaii Farm Bureau's State Farm Fair. Eight displays were produced for Maui headquarters and one for the Oahu office.

Slide collection:

A slide collection illustrating all facets of NifTAL's work was begun. Illustrative materials such as distribution maps, flow diagrams, and explanatory drawings were produced and photographed for the collection. An index to the collection was also produced to assist staff members in finding support materials.

Four copies of a 75-slide presentation entitled "What is NifTAL?" were produced. These can be borrowed by staff members who are travelling. The presentation is arranged so that any part of it can be used alone for shorter talks, and so that the individual staff member can augment the basic sets with slides of his own. This slide show has been used on six trips to date and all or portions of it have been viewed at 15 institutions in Asia, SE Asia, the Pacific, USA, South America, Europe, Middle East, and Africa.

Distribution:

At the end of FY 1978/79 NifTAL had a mailing list of over 600 scientists, students, libraries, and institutions. To this core list, over 300 additional names have been added. A number of sources have been researched for names and addresses of scientists and administrators in NifTAL's client group: participants at BNF-related symposia, directories, mailings lists of related research projects and of the Publications and Information Office of CTAHR. The list is now being scrutinized by Regional Coordinators, Consortium contract directors, and knowledgeable administrators to determine what types of information (*e.g.* scientific, news-oriented, announcements) are appropriate for each potential recipient. Names are also screened for continuing interest in BNF research. Each entry on the current list has been coded for geographical area and client category.

See Appendix 5 for distribution statistics on NifTAL publications.

NifTAL Project

Forecast of Work Plan for Coming Year
(July, 1980 - June, 1981)

ACTIVITY	SCOPE OF WORK	OUTPUT
NETWORK	Continue and intensify effort on establishment of INLIT Expt. A at up to 200 field locations in developing countries.	Confirmed cooperators. Trial results.
	Development of memoranda of understanding with foreign institutions as and when required and involving AID Missions to the extent possible.	Memoranda of understanding.
	Integrate INLIT to investigations at Benchmark Soils Project sites.	Memoranda of understanding.
	Develop statistical analysis and computer program for INLIT data handling.	Addition to INLIT manual.
	Develop protocol for Expt. B.	Addition to INLIT manual.
	Develop protocol for Expt. C.	Addition to INLIT manual.
	Participate in meetings/workshops to create awareness of INLIT program and offer assistance to other agencies in the development of national and international programs in BNF technology.	Duty travel completed. Trip report.
	Organization of an international workshop on applied research in BNF technology as an occasion for coordination of INLIT program and integration with other related projects.	1st and 2nd announcements. Program. Book of Abstracts. Conference Proceedings (Nov., 1981).
	Develop antisera for back-up services to INLIT cooperators.	Limited serum bank.
	Initiation of an international Bulletin carrying highlights of applied BNF research training/service programs worldwide to range of clients, primarily non-technical.	BNF Bulletin.

ACTIVITY	SCOPE OF WORK	OUTPUT
NETWORK (contd)	Provision of technical documents and information to requestors from LDCs.	Answered requests.
TRAINING	Organize and mount a Training Course in Legume/*Rhizobium* Technology in cooperation with the Colegio de Postgraduados, Chapingo, Mexico.	Thirteen trainees in Latin American region.
	Develop Spanish translation of Training Manual.	Publication in Spanish.
	Initiate discussions on 1982 course to be held at Kasetsart, Bangkok, Thailand.	Agreement with host institution.
	Amplify material in the Training Manual.	Expanded Training Manual.
	Identify key individuals requiring BNF training and offer non-degree intern programs in Hawaii.	Twelve trainees.
	Offer up to five graduate assistantships to LDC and U.S. students enrolled in U.H. and committed to undertaking research in furtherance of NifTAL's contracted objectives.	M.S. and Ph.D. graduates.
	Produce and distribute information on training opportunities with NifTAL and activities of former trainees.	Brochure. Newsletter.
	Obtain skills in preparation of audiovisual teaching aids.	Improved training capability.
	Production of set of audiovisuals on basic techniques for applied research on BNF and field testing/evaluation of same.	Audiovisual modules (6).
	Perform an evaluation of the impact of BNF technology training in agricultral research in developing countries during 1970-1980.	Report.

ACTIVITY	SCOPE OF WORK	OUTPUT
TRAINING (contd)	Offer training support to courses sponsored by other institutions; specifically: --UNEP/Unesco/ICRO course on Culture Collections at Ainshams University, Cairo, Egypt. --INIA/NCSU Training Course, La Molina, Lima, Peru. --UNEP/Unesco/ICRO Training Course at TISTR, Kasetsart, Thailand. --UNDP/IITA, Ibadan, Nigeria.	
GERMPLASM (a) Rhizobial	Consolidate the Rhizobium Germplasm Resource at its present scale (1,500) and conduct a revision of the collection for viability and purity prior to freeze-drying.	High quality collection.
	Perform isolations in support of INLIT cooperators, staff technical assistance to foreign institutions and to others to an extent that is reasonably practicable (no commitment to preserve or evaluate such strains unless by special arrangement).	Accommodated requests. Rhizobium cultures.
	Characterize strains by standardized traits.	Data.
	Continue freeze-drying culture collection.	Assured perpetuation of collection.
	Computerize strain information.	Accessible collection.
	Develop, produce and distribute a catalogue of strains.	Catalogue.
	Authenticate strains as Rhizobium in tubes under growth room conditions.	Authenticated strains.

ACTIVITY	SCOPE OF WORK	OUTPUT
GERMPLASM (a) Rhizobial (contd)	Test authenticated strains for potential nitrogen fixation effectiveness in Leonard jars under greenhouse conditions.	Potentially effective strains.
	Test potentially effective strains in pots of field-collected soil under greenhouse conditions to examine their effectiveness under simulated field conditions.	Pot-tested potentially effective strains.
	Test potentially effective, pot-tested strains in the field at sites in Hawaii and elsewhere in the tropics to examine strain effectiveness under local soil and climatic conditions.	Field proven collective strains.
	Test potentially effective, pot-tested strains in the field at sites in Hawaii and elsewhere in the tropics to examine strain effectiveness under local soil and climatic conditions.	Network trials. (INLIT)
	Maintain a current listing of strains for use with contract legumes with basis for recommendations defined.	Brochure.
	Design, construct and operate a pilot production plant appropriate for LDC circumstances.	Pilot plant.
	Conduct research to simplify and reduce the cost of inoculant production.	Improved technology.
	Provide inoculants to researchers in LDCs.	Accommodate requests.
(b) Leguminous	Test genotypic variation among host/Rhizobium combinations for tolerance to soil stresses common in the tropics, and focussing on improved legume/Rhizobium symbioses tolerant of (a) transient excesses of soil nitrate; (b) acid soil stresses; and (c) fragile environments with protracted dry seasons.	Improved TA capability.

BUDGET FORECAST

Estimated Expenditures and Obligations

July 1, 1980 - June 30, 1981

According to Inputs:

Salaries/Wages	$375,350
Fringe Benefits	24,853
Consultants	3,990
Network	75,000
Travel/Subsistence	34,091
Participants	75,000
Freight	518
Publications	7,764
Equipment/Supplies	84,002
Subcontract	38,711
Vehicle Lease	2,103
Overhead	99,659
TOTAL	$821,041

According to Outputs:

Network	410,520
Training	205,260
Germplasm	82,104
Inoculant technology	82,104
Delivery technology	41,053
TOTAL	$821,041

STATEMENT OF EXPENDITURES AND OBLIGATIONS
AND CONTRACTOR RESOURCES
1979 - 1980

EXPENDITURES AND OBLIGATIONS BY INPUT

Category	Amount
Salaries and Wages	$363,545.00
Fringe Benefits	48,766.00
Consultants	4,835.00
Network Costs	27,135.00
Travel & Subsistence	90,702.00
Participant Costs	33,621.00
Freight	1,528.00
Publication	8,294.00
Equipment & Supplies	173,898.00
Subcontract	36,091.00
Vehicle Lease	4,308.00
Overhead	92,663.00
Grand Total	$885,386.00

ESTIMATED EXPENDITURES AND OBLIGATIONS BY OUTPUT

Network	$442,693.00
Training	221,346.00
Germplasm	88,539.00
Inoculant Technology	88,538.00
Delivery Technology	44,270.00
Total	$885,386.00

APPENDIX 1

RESEARCH TABLES
AND FIGURES

Figure 1. Summary of the procedure for dilution technique.

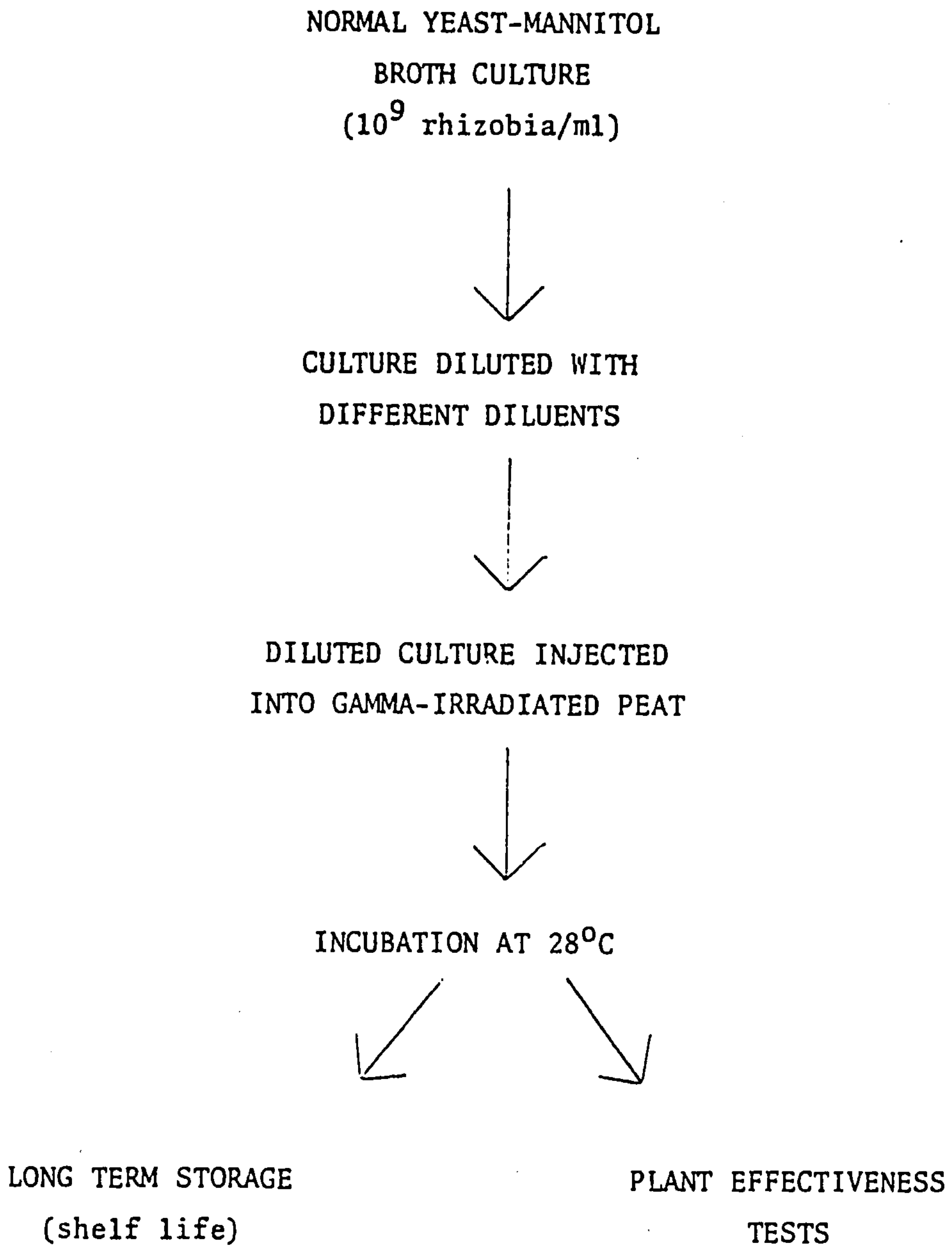


Table 1. Influence of initial inoculum level on the subsequent growth and survival of *R. phaseoli* in gamma-irradiated peat at 28°C.

*Level of diluted broth inoculum (log. no. of viable cells per ml)	Log. no. of viable cells of *Rhizobium* per g. peat after (weeks)							
	0	1	2	3	4	6	8	10
6.00	4.95	9.72	10.25	10.11	9.97	9.97	9.88	10.07
7.00	6.25	9.87	10.15	9.95	9.91	10.03	9.75	9.63
8.00	7.43	10.00	10.05	9.95	9.85	10.16	9.67	9.69

*25% yeast-mannitol broth was used as diluent.

Table 2. Influence of initial inoculum level on the subsequent growth and survival of *R. japonicum* (TAL 102) in gamma-irradiated peat inoculated at 28°C.

*Level of diluted broth inoculum (log. no. of viable cells per ml)	Log. no. of viable cells of per g. peat after (weeks)							
	0	1	2	3	4	6	8	10
6.00	5.42	9.96	10.14	10.17	9.98	10.11	10.04	9.83
7.00	6.31	10.06	10.25	10.09	9.98	10.13	10.08	9.99
8.00	7.45	10.18	10.11	10.10	10.03	10.17	10.00	9.77

*25% yeast-mannitol broth was used as diluent.

Table 3. Effect of different diluents on the growth and survival of *R. phaseoli* (TAL 182) during long-term storage at 28°C in Australian peat.

Diluents	Log. no. of viable cells per g. peat after (days)						
	0	14	30	60	90	120	Means
Sterile deionized water	4.46	9.75	9.69	9.36	8.89	8.67	8.47
25% yeast mannitol broth	4.38	9.98	9.89	9.58	8.93	8.47	8.54
25% yeast water	4.43	9.69	9.49	9.44	8.81	8.60	8.41
25% yeast sucrose broth	4.31	9.98	9.79	9.60	9.19	8.67	8.59
Means	4.39	9.85	9.72	9.49	8.95	8.60	

Diluents: N.S.

Time: Bayes LSD (0.05) 0.15

Table 4. Effect of different diluents on the growth and survival of *R. japonicum* (TAL 102) during long-term storage at 28°C in Australian peat.

Diluents	Log. no. of viable cells per g. peat after (days)						
	0	14	30	60	90	120	Means
Sterile deionized water	4.32	10.01	9.83	9.73	9.43	8.65	8.66
25% yeast mannitol broth	4.41	10.06	9.96	9.93	9.60	8.74	8.78
25% yeast water	4.37	9.81	9.88	9.77	9.63	8.81	8.71
25% yeast sucrose broth	4.53	9.94	9.98	9.91	9.46	9.08	8.82
Means	4.41	9.96	9.91	9.84	9.53	8.82	

Diluents: N.S.

Time: Bayes LSD (0.05) 0.17

Table 5. Effectiveness of inoculants prepared with the different diluents and Australian peat after 3 months of storage at 28°C.

Diluent	*Mean dry weight of plant tops (g)	
	P. vulgaris[1]	*G. max*[2]
Sterile deionized water	2.49	4.38
25% yeast mannitol broth	2.24	4.76
25% yeast water	2.24	5.16
25% yeast sucrose broth	2.17	5.28
	NS	NS

*values of 6 replicates with 2 plants per replicate.

[1, 2] *P. vulgaris* and *G. max* were inoculated with TAL 182 and TAL 102 prespectively.

Table 6. Strains for use in Experiment A.

LEGUME SPECIES	TAL #	ORIGINAL DESIGNATION(S)
Arachis hypogaea	1000	NifTAL 1000
	169	176A22 (Nitragin)
	1371	T1
Centrosema pubescens	651	UMKL 44
	655	UMKL 09
	1146	CIAT 590
Cicer arietinum	620	ICRISAT 3889
	480	UASB 67
	1148	27A3 (Nitragin)
Desmodium uncinatum	569	SPRL 472
	1147	CIAT 299
	667	CIAT 13; SPRL 471
Glycine max	102	USDA 110
	377	USDA 138
	379	CB 1809; USDA 136b
Lens culinaris	634	B-13; NZP-5400
	638	I-2
	640	I-11
Leucaena leucocephala	82	TAL 82
	1145	CIAT 1967
	582	CB 81
Medicago sativa	380	SU 47
	1372	POA 116
	1373	POA 135
Phaseolus vulgaris	182	NifTAL 182
	613	CIAT 57; CC 511
	1121	127K17 (Nitragin)
Stylosanthes guianensis	309	CB 756
	310	CB 1024
	658	CIAT 71
Vigna radiata	441	M 6
	420	THA 301
	169	176A22 (Nitragin)
Vigna unguiculata	209	TAL 209
	173	176A30 (Nitragin)
	658	CIAT 71

Figure 2. Synchronous cultures of CB 746 with and without acid/Al stress. Conditions were imposed 3 days before establishment of synchrony, and maintained throughout. Lines subjectively fitted.

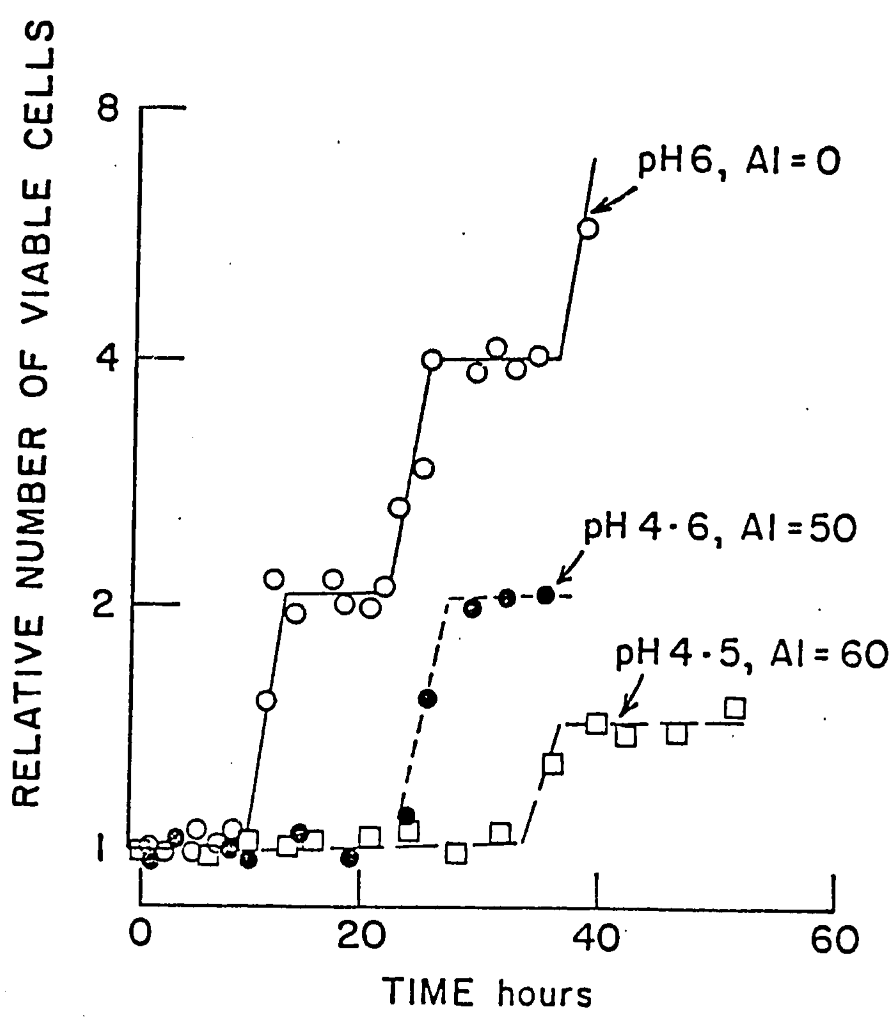


Figure 3. Similarity of growth response to acid/Al stress regardless of prior exposure to the stress. Cultures of TAL 189 had been previously subcultured in either acid/Al or no-stress media, to a maximum of 9 serial transfers (190 days, estimated 72 generations).

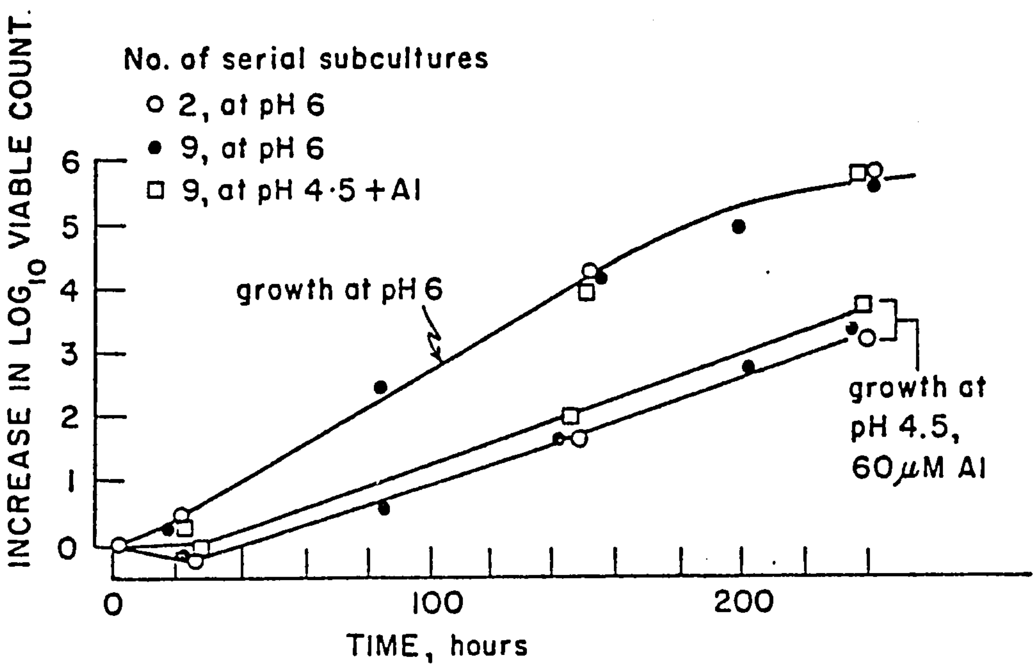


Table 7. Effect of source of isolates on their ability to colonize CGAAL medium (acid citrate glutamate agar with ($Al(OH)_3$) at pH 4.5.

	$\log_{10}$ (colonies produced/cells inoculated)			
Strain	from YMA pH 6.5	from CGA pH 6.2	from CGAAL pH 4.9	from CGAAL pH 4.5
316n9	< -7	< -6	< -6	nd
TAL189	-2.0	-2.2	nd	-2.7
CB756	-1.0	-1.0	nd	-0.7
TAL425	-1.5	-0.6	nd	-0.5
TAL171	-0.8	nd	nd	-0.6
LSD p = 0.05	1.05			

(316n9 excluded from analysis of variance)

Table 8. Growth of 6 *Rhizobium japonicum* strains in a limonite dialysis culture medium buffered at two phosphorus concentrations.

Strain	Initial cell density (cells/ml)	Generations after 76 hours growth 4×10^{-7} M P	7×10^{-6} M P
USDA 138	940	6.9*	9.3
USDA 110	910	6.4	6.5
USDA 6	840	5.3	8.4
USDA 136	1220	4.8	5.7
USDA 122	1280	4.6	5.6
USDA 142	1170	2.4	3.9

*L.S.D. (P=0.05) for comparison of means of one strain at different P levels or strain differences at the same P level are 0.5 and 0.3, respectively.

Table 9. The effect of inoculation and fertility level on yield of green beans, chlorophyll content of leaves, and acetylene reduction of roots of *Phaseolus vulgaris*, var. 'Bountiful'.

TREATMENTS		Yield green beans (kg/plot)	Leaf chlorophyll content (mg/g fresh leaves)	Acetylene reduction of nodulated roots (μM/plant/hr.)
Fertility	Inoculation			
Maximum				
	Uninoculated	5.50 b*	.62 a	2.91 ab
	Uninoc. + N	7.20 c	1.10 b	.41 a
	Inoculated	6.60 bc	.98 b	11.09 c
		6.43 = Mean	.90 = Mean	4.80 = Mean
Farm				
	Uninoculated	2.57 a	.38 a	.08 a
	Uninoc. + N	5.80 bc	.94 b	.05 b
	Inoculated	3.25 a	.94 b	6.45 bc
		3.87 = Mean	.75 = Mean	2.19 = Mean

*Means followed by the same letter in each column are not significantly different at the 5% level of probability by Duncan's Multiple Range Test.

Table 10. The effect of inoculation and fertility level on yield of seed, chlorophyll content of leaves, and acetylene reduction of roots of *Glycine max*, var. 'Davis'.

TREATMENTS		Yield of seed (kg/ha)	Leaf chlorophyll content (mg/g fresh leaves)	Acetylene reduction of roots (μM/plant/hr.)
Fertility	Inoculation			
Maximum				
	Uninoculated	1120 ab*	14.0 a	0.1 a
	Uninoc. + N	1370 ab	23.8 b	0.1 a
	Inoculated	1840 b	25.2 b	7.8 b
		1440 = Mean	21.2 = Mean	2.7 = Mean
Farm				
	Uninoculated	870 a	16.2 a	0.1 a
	Uninoc. + N	1050 ab	25.5 b	0.1 a
	Inoculated	630 a	24.9 b	7.0 b
		850 = Mean	22.2 = Mean	2.4 = Mean

*Means followed by the same letter in each column are not significantly different at 5% level of probability by Duncan's Multiple Range Test.

Table 11. Mid-flowering data, Chickpea, Experiment A.

	Uninoculated	Uninoculated + 100 kg N/ha	Inoculated
Farm Fertility Level			
g dm in tops/m of row	89.4 ± 33	55.1 ± 7.3	123.2 ± 31.4
g dm/plant top	6.02 ± 2.9	4.78 ± .88	8.93 ± 1.80
High Fertility Level			
g dm in tops/m of row	82.3 ± 23.4	82.3 ± 23.4	138.55 ± 34.4
g dm/plant top	7.04 ± 2.33	7.04 ± 2.33	11.35 ± 1.80

Four replications per treatment. Values are means ± S.D.'s.

Figure 4.

Effect of a continuous high level of KNO_3 on acetylene reduction rate of Davis soybean infected with different *Rhizobium* strains. 102 and 379 in preliminary experiments appeared to suffer less from NO_3^- stress. Plants in sand culture watered with nutrient 2x daily 20 days old at time zero.

SYMBOL	STRAIN
+	TAL 102
x	TAL 630
o	TAL 379
●	TAL 633

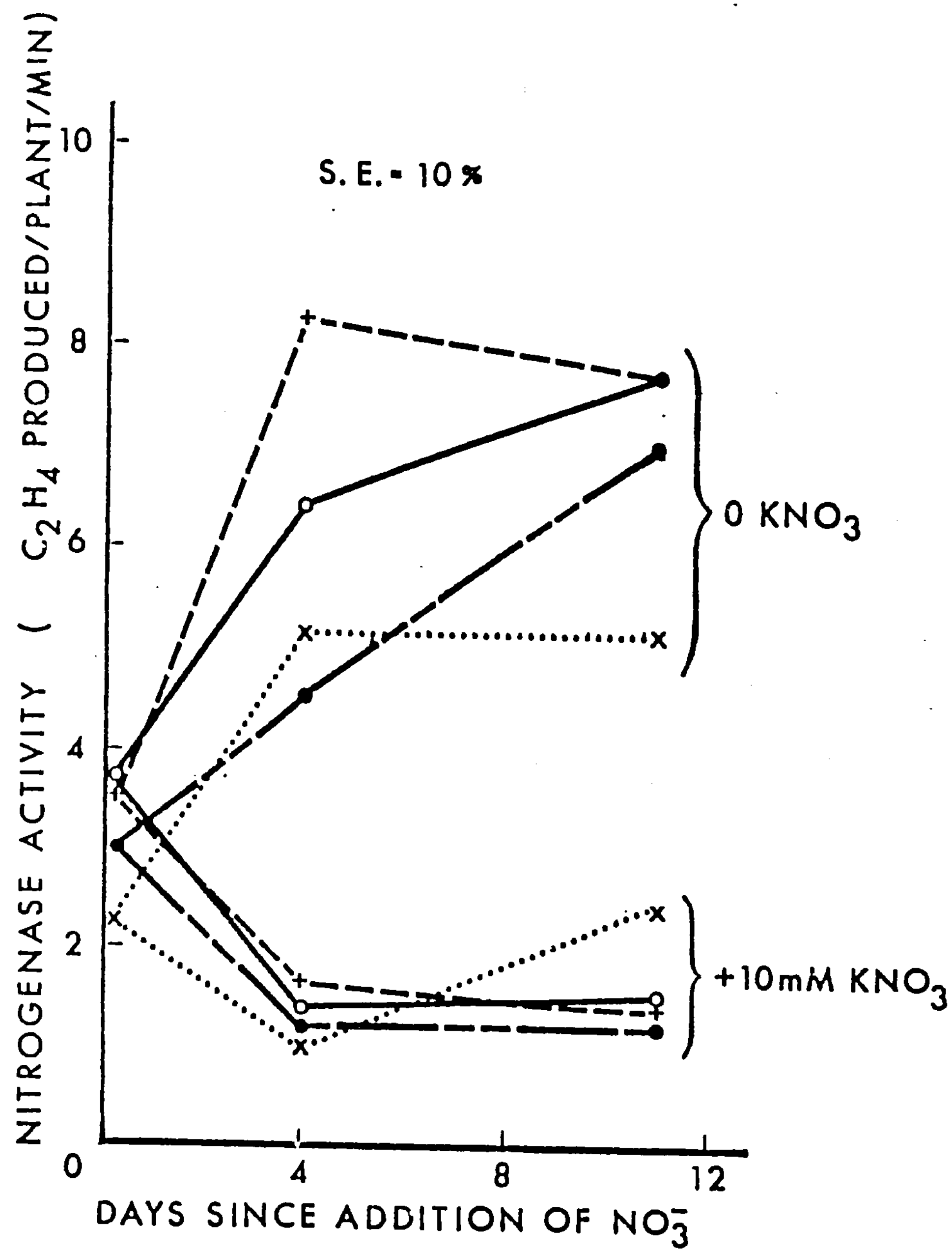


Table 12. The effect of NO_3^- concentration dry matter production of the tops, root nodules, acetylene reduction, and nodule number of soybeans (*Glycine max*, var. 'Davis').

Concentration of NO_3^- in sol'n (mM)	Nodule number (per plant)	Dry matter (g./pl.)			C_2H_2 Reduction (M/hr/pl)
		Tops	Roots	Nodules	
0	90	0.94 a**	0.18 a	.137	11.2 b
0.5/0*	178	3.06 b	1.29 d	.062	6.2 ab
0.5	12	4.23 c	0.86 b	.014	1.0 a
1.0	3	4.73 c	1.15 c	---	---
2.0	3	4.61 c	1.08 c	---	---

* Concentration maintained at 0.5 mM for 14 days, then reduced to 0 for next 8 days.
**Means followed by a common letter in the same column are not significantly different at the 5% level.

Table 13. The effect of the cultural method and nitrate nitrogen supply on dry weight of tops and roots, nodule mass, and acetylene reduction of soybean (*Glycine max*).

Cultural method	Nitrate (mM NO_3^-)	Wt. of tops (g./pl.)	Wt. of roots (g./pl.)	Nodule mass (g./pl.)	Acetylene reduction (μM/pl./hr)
Solution	0	1.59 a*	.33 a*	.163 b	13.9 bc
Gravel	0	1.55 a	.46 ab	.175 b	16.9 c
Sand	0	1.61 a	.21 a	.172 b	16.8 c
Solution	1.0	6.23 d	1.53 d	.010 d	0.6 a
Gravel	1.0	3.91 c	.86 c	.086 c	11.2 b
Sand	1.0	2.60 b	.55 b	.256 a	34.2 d

*The means followed by a common letter in the same column are not significantly different at the 5% level.

Table 14. Effect of nitrate on number of nodules per single root.

Concentration of mM NO_3 on:			Number of nodules per single root.					
Treatment #	Main Roots	Single Roots	Replications					Mean
			1	2	3	4	5	
1	0	0	2	0	28	7	12	9.9
2	1	0	0	0	0	10	0	2.0
3	0	1	7	1	5	2	2	3.7

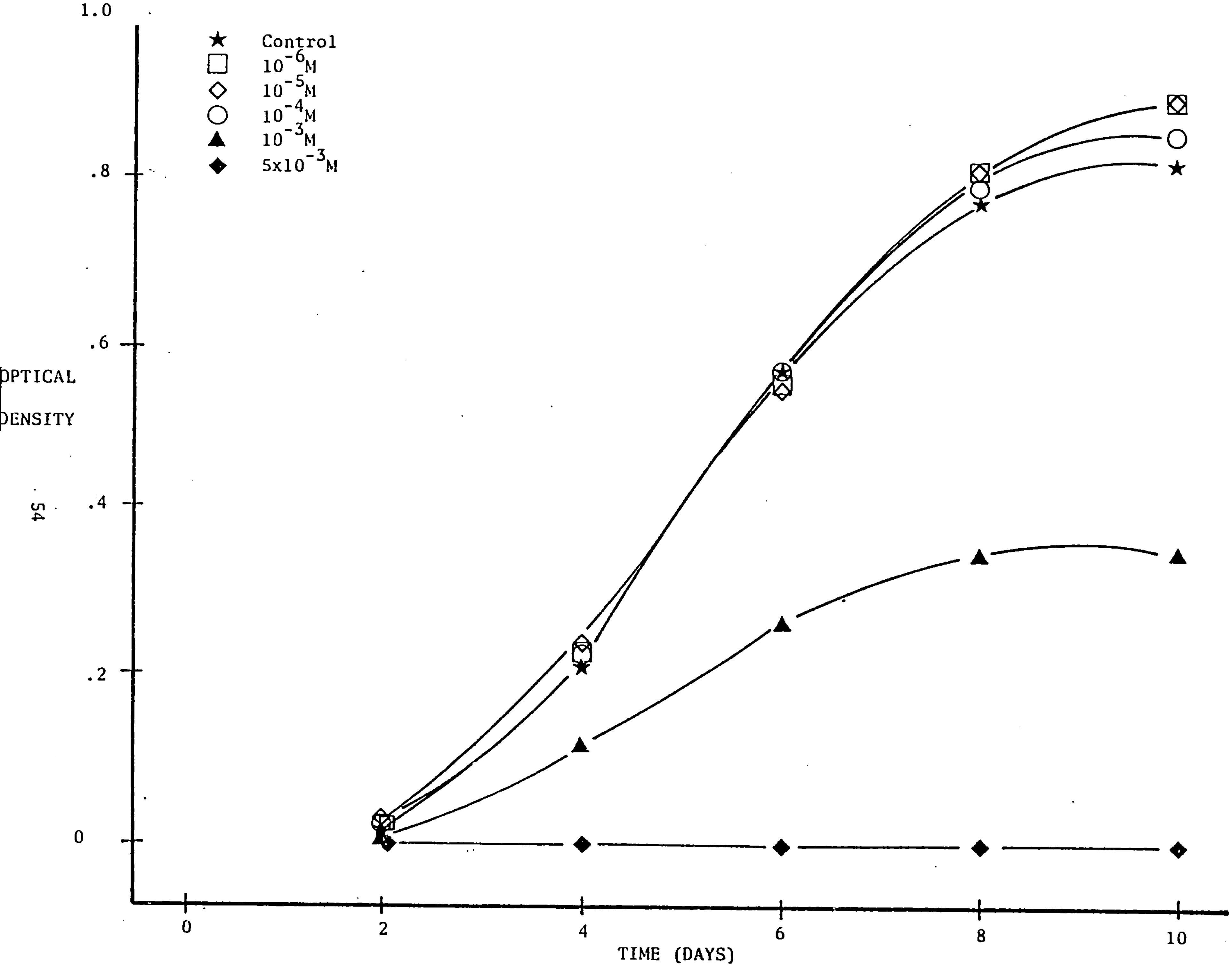


Figure 5. Growth of *R. meliloti* (TAL 380) at differing concentrations of p-Coumeric acid.

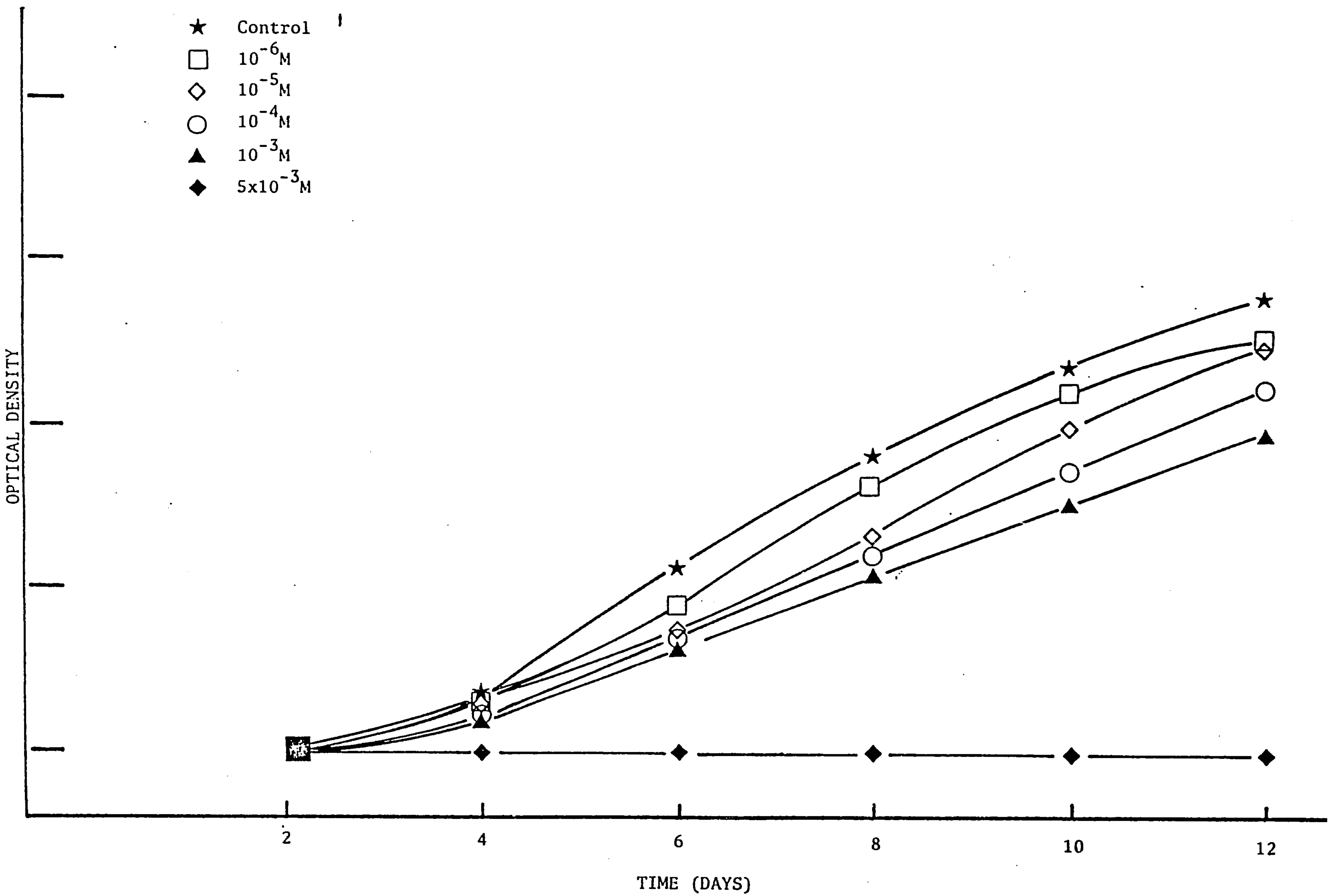


Figure 6. Growth of *Rhizobium* sp. (CIAT 299, a *Desmodium intortum* isolate) at differing concentrations of p-Coumeric acid.

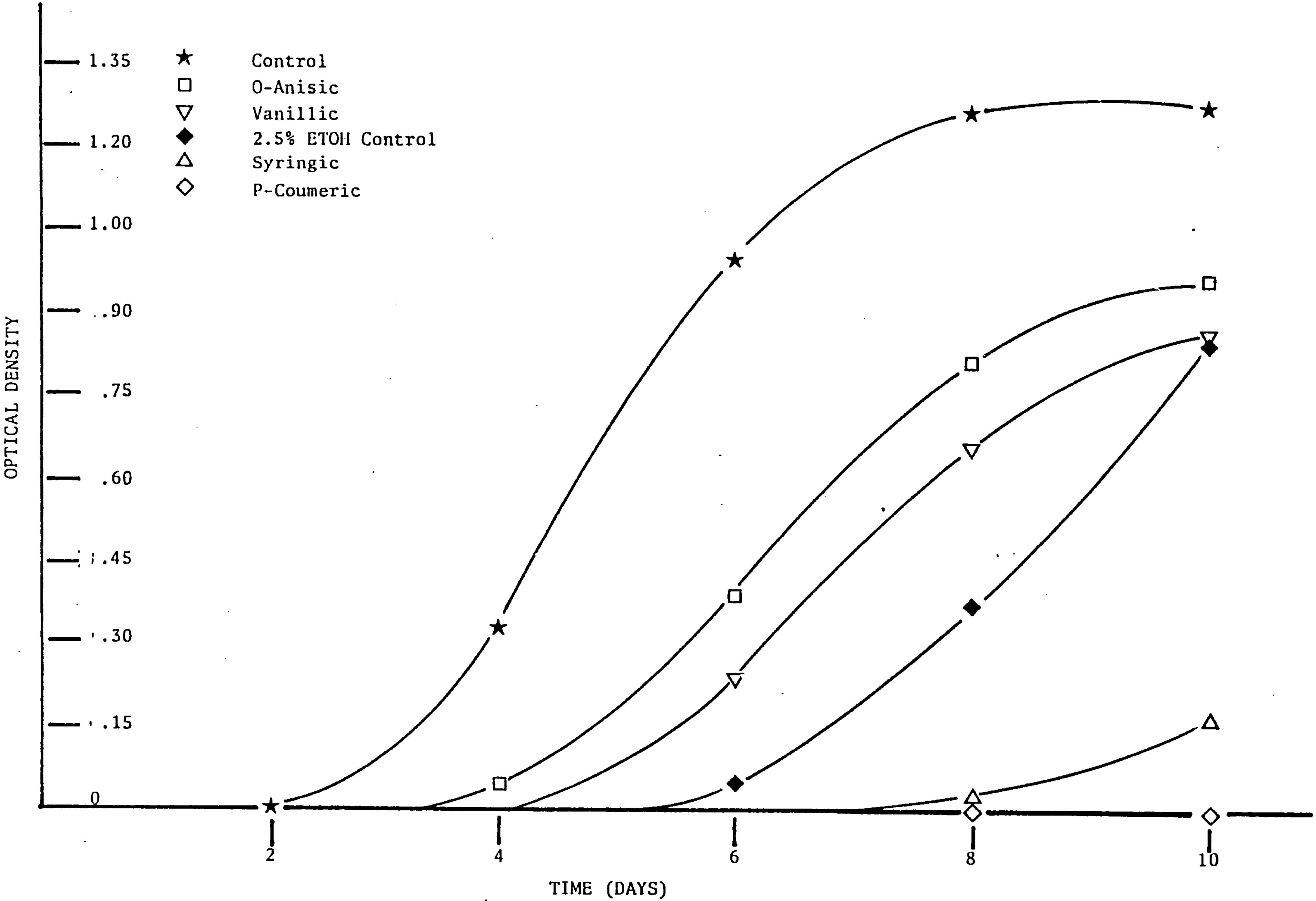


Figure 7. Growth of TAL 620 (*R. leguminosarum*) in the presence of neutralized phenolic acids at 5×10^{-3} M concentration.

APPENDIX 2

AGROFORESTRY QUESTIONNAIRE

N_2-FIXING TREES FOR AGROFORESTRY SYSTEMS

Questionnaire

A letter from Jake Hlliday was enclosed with each questionnaire and included the following information:

"We are appraising a wide range of tree species as the first step in a project on 'Nitrogen-Fixing Trees for Agroforestry Systems,' a joint ICRAF/NifTAL Project. These trees will be characterized for *Rhizobium* requirements, nodulation, nitrogen fixation, and whether they develop mycorrhizal associations. The trees will be field tested at a number of locations to define their range of adaptation. Fast-growing trees emerging from this program will be tested in Thailand as a component in our cooperative project with the Royal Forestry Department on 'Establishment of Leguminous Trees in Deforested Areas of Thailand.'

"I believe you may be able to provide us with information on some of the species on the list appended to this letter. You may also be able to suggest potentially useful species not as yet included in this list of species being given priority in our project. Most importantly, you may be able to provide seed, or indicate a source of seed, of the species listed. Please send us details, including cost, of any seed you have available. Any information relating to germination and seedling culture that you have would obviously be of great value to us."

SPECIES TO BE APPRAISED FOR AGROFORESTRY SYSTEMS

Please complete and return to:

Dr. J. Halliday
Director, NifTAL Project
University of Hawaii
P. O. Box "O"
Paia, Hawaii, 96779, U.S.A.

Species no.	Scientific name	Common name known to you	Do you have seed? (Yes No)	Comments (distribution, nodulation, references) --use separate sheet, if necessary--
1	*Acacia albida*			
2	*A. senegal*			
3	*A. nilotica*			
4	*A. tumida*			
5	*A. mearnsii*			
6	*A. holosericea*			
7	*A. pyrifolia*			
8	*A. linaroides*			
9	*A. bivenosa*			
10	*A. seyal*			
11	*A. cyanophylla*			
12	*Alnus acuminata*			

Species no.	Scientific name	Common name known to you	Do you have seed? (Yes/No)	Comments (distribution, nodulation, references) --use separate sheet, if necessary--
13	*Prosopis chilensis*			
14	*P. juliflora*			
15	*P. cineraria*			
16	*P. africana*			
17	*P. tamarugo*			
18	*Parkia biglobosa*			
19	*Parkia filicoidea*			
20	*Tamarindus indica*			
21	*Casuarina equisetifolia*			
22	*Afzelia africana*			
23	*Cordia alliodora*			
24	*Cordeauxia edulis*			
25	*Azadirachta indica*			
26	*Albizia* spp.			
27	*Julbenardia globiflora*			
28	*Terminalia* spp.			
29	*Brachylaena hutchinsii*			
30	*Sesbania grandiflora*			

Species no.	Scientific name	Common name known to you	Do you have seed? (Yes No)	Comments (distribution, nodulation, references) --use separate sheet, if necessary--
31	*Combretim* spp.			
32	*Leucaena leucocephala*			
33	*Cassia spectabilis*			
34	*Cassia siamea*			
35	*Brachystegia spiciformis*			
36	*Acacia auriculiformis*			

APPENDIX 3

NifTAL TRAINING

Alphabetical List of NifTAL-Supported Graduate Students (1975 - 1980)

Student	Status	Degree	Date of completion
Michael Akyeampong	completed	M.S.	1980
Ken Cassman	completed	Ph.D.	1979
Shiva Chaudhary	in progress	Ph.D.	1981
Stephen Dowdle	to begin 9/80	Ph.D.	
Flemming Eriksen	completed	Ph.D.	1978
Sheila May	completed	M.S.	1979
Marcelo Sagardoy	to begin 9/80	Ph.D.	
Paul Singleton	completed	M.S.	1979
William South	in progress	M.S.	1981
Paul Woomer	completed	M.S.	1979

Alphabetical List of NifTAL Intern Trainees (FY 1979 - 1980)

Student	Country
Ashar Al-Nashi	Iraq
Pornpun Jongsuksuntigool	Thailand
Jessada Kaewchote	Thailand
G. Bhaskar Reddy	India
Tarlochan Singh Sandhu	India
Nuno M. de Sousa Costa	Brazil

Alphabetical List of Trainees at NifTAL/MIRCEN Nairobi Course

Trainee	Country
Sayeda A. Abu Nayib	Sudan
John O. Ekpe	Nigeria
Mohamed Ahmed Abd. El Daiem	Egypt
M. S. Elegba	Ghana
Athanase Hakizimana	Rwanda
N. A. Hegazi	Egypt
G. R. Mathenge	Kenya
G. P. Msumali	Tanzania
F. Njeru	Kenya
R. C. Nyemba	Zambia
Alfred Traore	Upper Volta
S. Wangaruro	Kenya

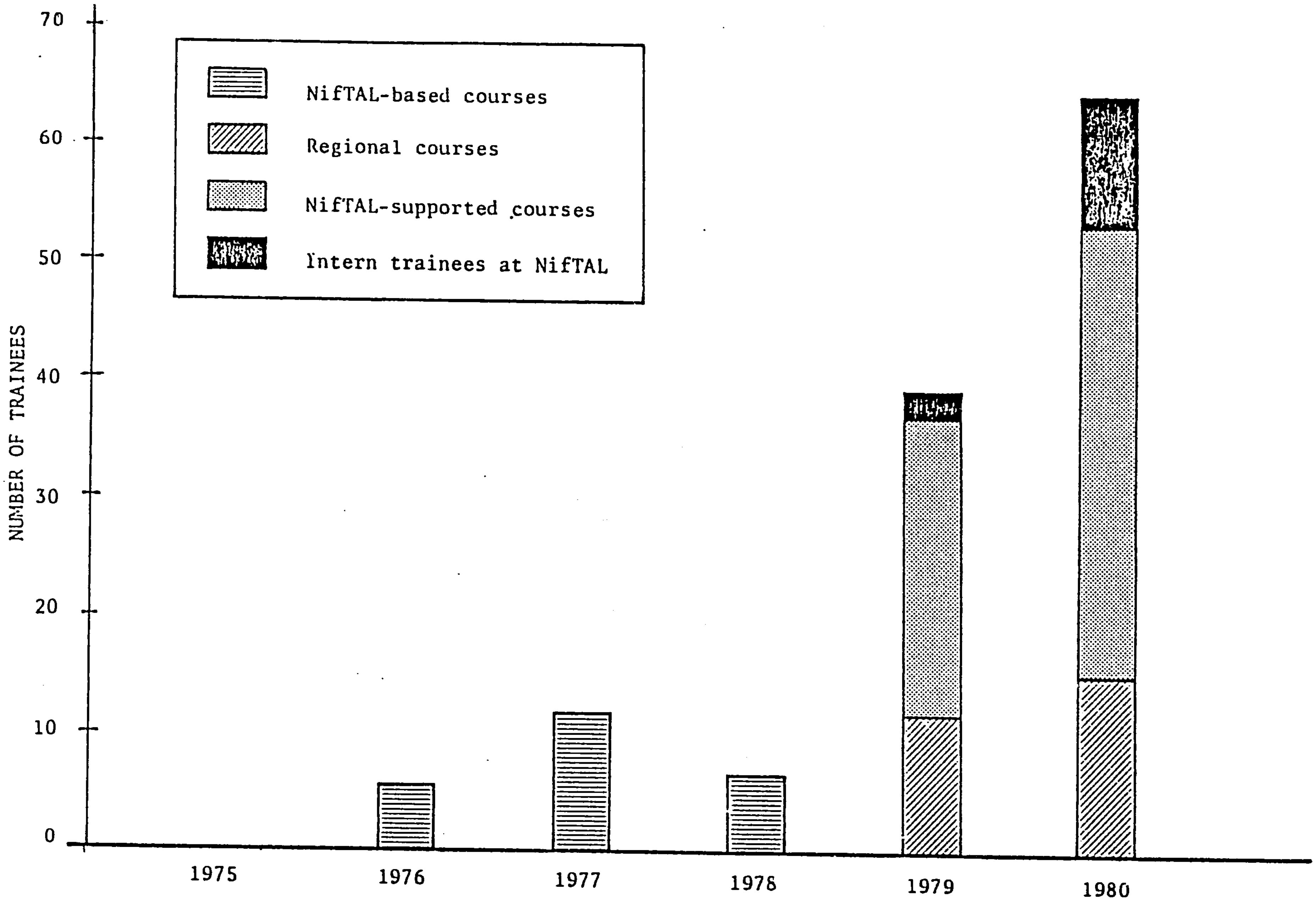


Figure 1. Number of trainees and distribution by type of training supported by NifTAL (1975-1980).

NifTAL/MIRCEN Training Course: Post-Session Survey

At the end of the Nairobi course, all participants were given a questionnaire to help the training staff in evaluating the course. The percentage of participants giving each answer is indicated on the sample questionnaire on the next page. Note that some questions were not answered by every respondent.

The results of the questionnaire and the comments of the trainees were major factors taken into consideration when revision of the training manual was undertaken. Future trainees will be also given questionnaires at the end of their courses. Intern trainees are questioned in detail to aid in course evaluation, but are not given a formal questionnaire.

A more extensive survey of the effect of training in institutions of the tropical world is planned for FY 1980 - 1981. In this survey many trainees from U.S., foreign national, and international institutions will be asked to evaluate the effect of their training.

NifTAL/MIRCEN TRAINING COURSE

IN *RHIZOBIUM* TECHNOLOGIES

Post-Session Survey

In order to improve our training course in Rhizobium *technologies, and to design future training programs, we need your help. Please evaluate your recent training session by using the following questionnaire. Be frank and honest in your replies, and please add your own comments if you wish. We value your opinions, as they will help us upgrade our training program.*

Thank you,
NifTAL Training Staff

Please circle the answer of your choice. Add remarks if you wish.

1. *How much new and useful information did the course present?*

75% a) A significant amount

25% b) A moderate amount

c) Very little

2. *If you had the necessary equipment and materials, could you now set up and operate a* Rhizobium *strain selection program in your own institution?*

75% a) Definitely

25% b) Possibly

c) No

3. *Do you now have the skills to provide technical sypport to a production-oriented legume program?*

37.5% a) Definitely

62.5% b) Possibly

c) No

4. *The level of instruction at which the course began was:*

12.5% a) Too advanced

75% b) Just right

12.5% c) Too simple

5. *How did the other candidates in the course influence your progress?*

50% a) They helped

37.5% b) No influence

12.5% c) They hindered

6. *There were* (circle one):

25% a) Too many instructors

37.5% b) An appropriate number

37.5% c) Too few instructors

7. *Was the knowledge you gained from the course worth the time and effort you put into it?*

87.5% a) Yes

12.5% b) No

c) Undecided

8. *Was the training manual a useful guide to the exercises in the course?*

100% a) Yes

b) No

9. *Do you have access to the additional reading materials mentioned in the manual?*

25% a) Yes

75% b) No

10. *Will the training manual be a useful reference tool in your home lab?*

75% a) Definitely

25% b) Possibly

c) No

11. *What changes are needed in your view, to make the training manual more useful?* (Please list suggestions here):

More detail, more diagrams and illustrations, MPN tables, formulae for solutions, instructions for preparation of media, data processing instruction, alternative carriers, instructions on assembly of equipment.

12. *How well did the course lecturers communicate? (That is, could you hear, see, and understand them clearly?):*

62.5% a) Very well

25% b) Moderately well

c) Not well

13. *With regard to the teaching staff, was the proportion of international instructors to local instructors satisfactory to you?*

50% a) Yes, there was a good balance of local and international participation.

50% b) No, there should have been more participation by international instructors.

c) No, there should have been more participation by local instructors.

14. *Was language a problem in the lectures?*

12.5% a) Yes

87.5% b) No

15. *Was new information presented* (choose one):

25% a) Too quickly?

75% b) At a good rate?

c) Too slowly?

16. *In terms of interest and information content, how would you rate the lectures?*

62.5% a) Very effective

37.5% b) Moderately effective

c) Ineffective

17. *Were the lab procedures taught logically and in a manner that built upon your past experience and knowledge?* (Please rate below, listing instances in which the procedures were not taught effectively.)

50% a) Lab procedures were taught clearly and thoroughly.

37.5% b) There was a significant degree of confusion in the lab training.

c) Lab procedures were taught poorly and in a disorganized manner.

Comments/suggestions on lab training process:

Need better facilities.
Follow manual's sequence
Course synopsis (Daily) needed.

18. *Was the equipment adequate for the training course?*

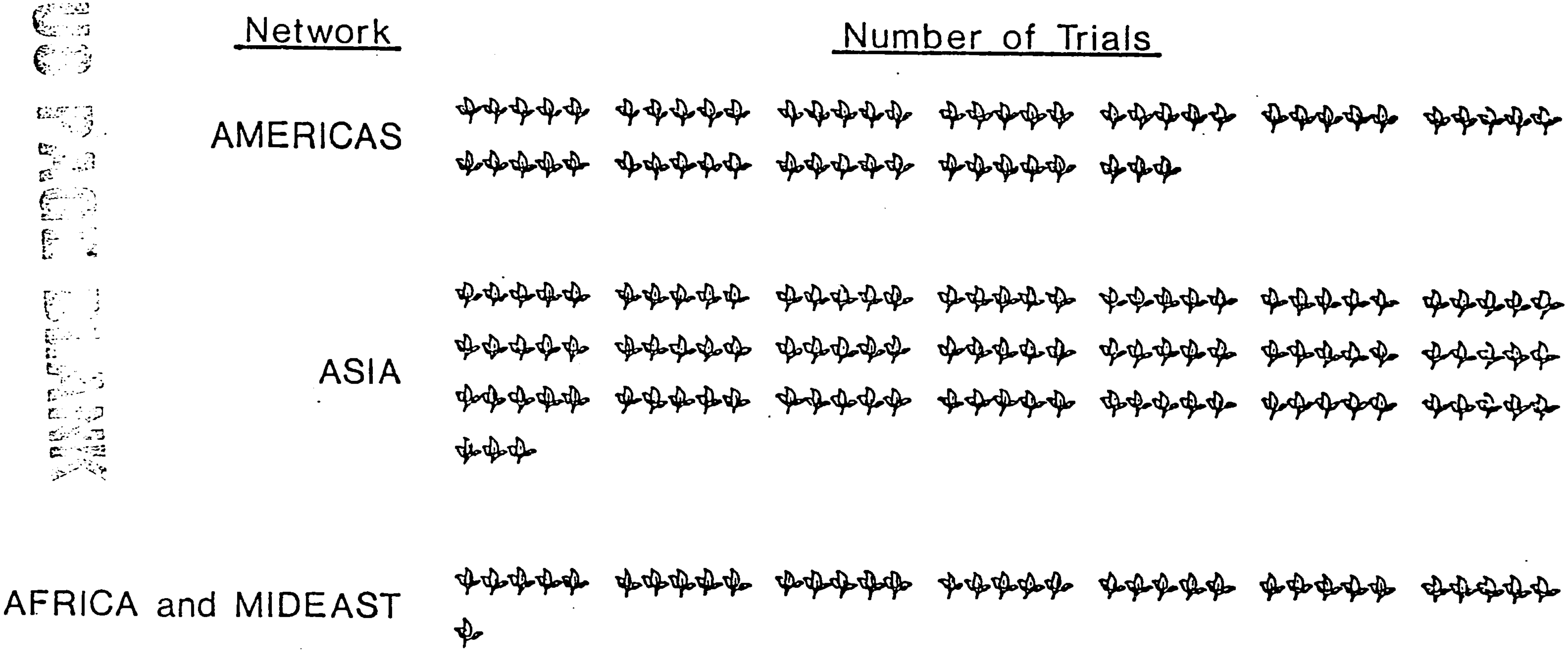
62.5% a) Yes

25% b) No

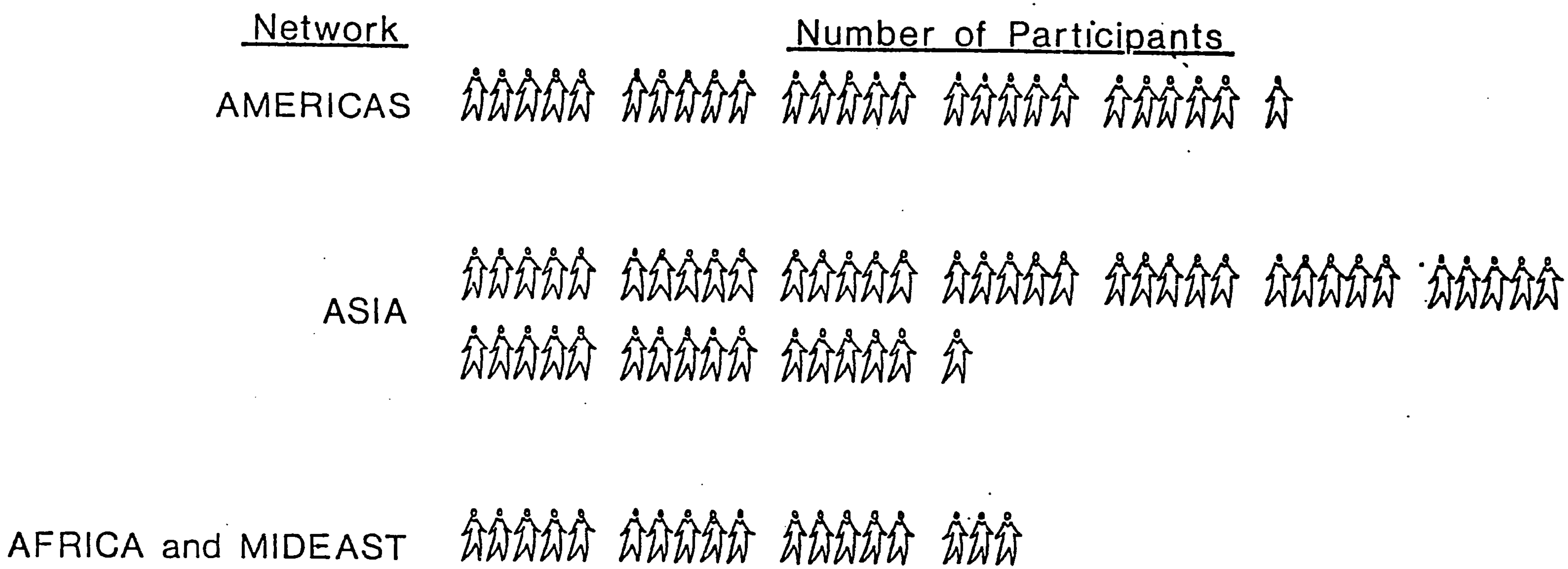
APPENDIX 4

NETWORK PARTICIPANTS

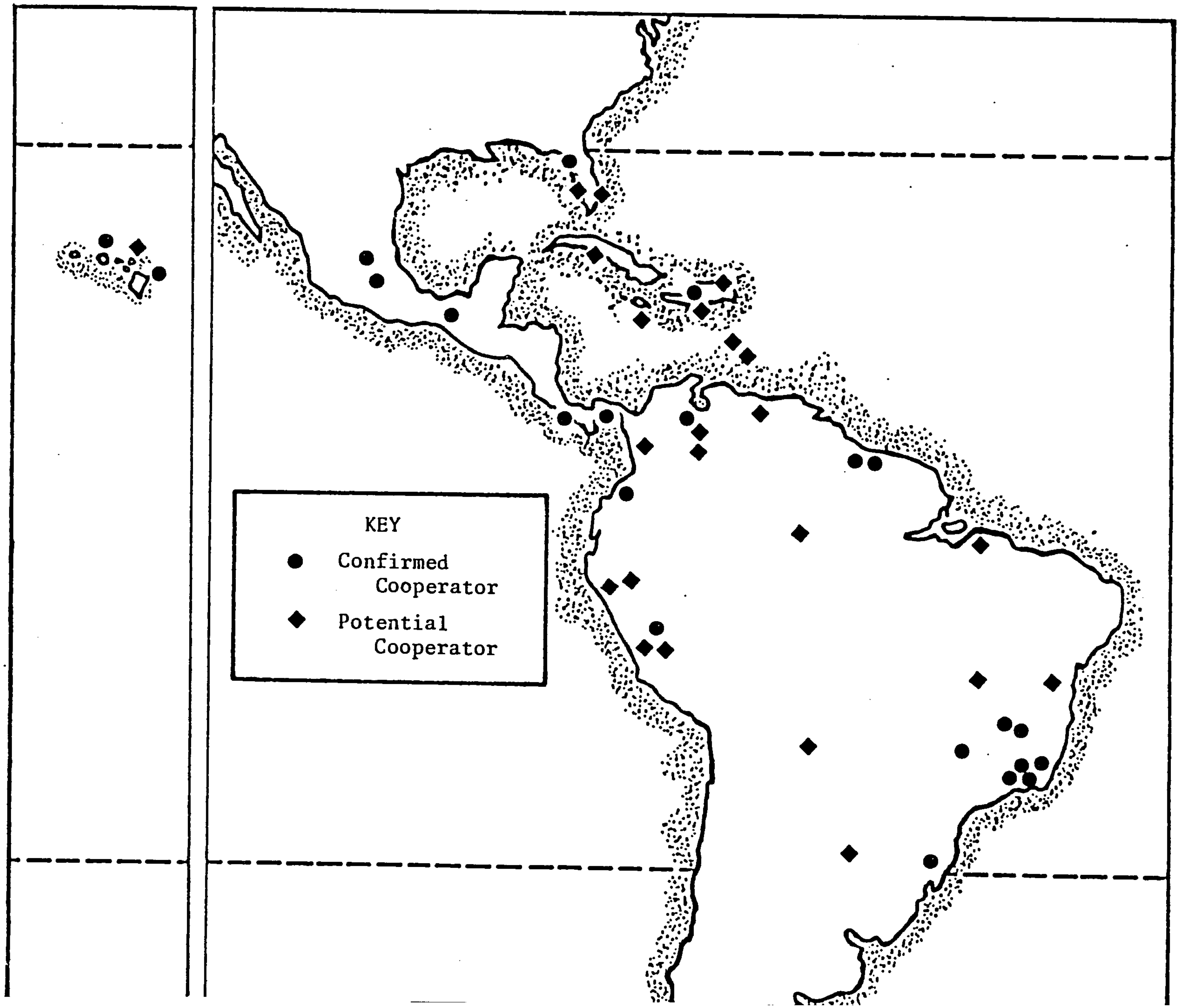
NUMBER OF TRIALS IN INTERNATIONAL NETWORK OF LEGUME INOCULATION TRIALS

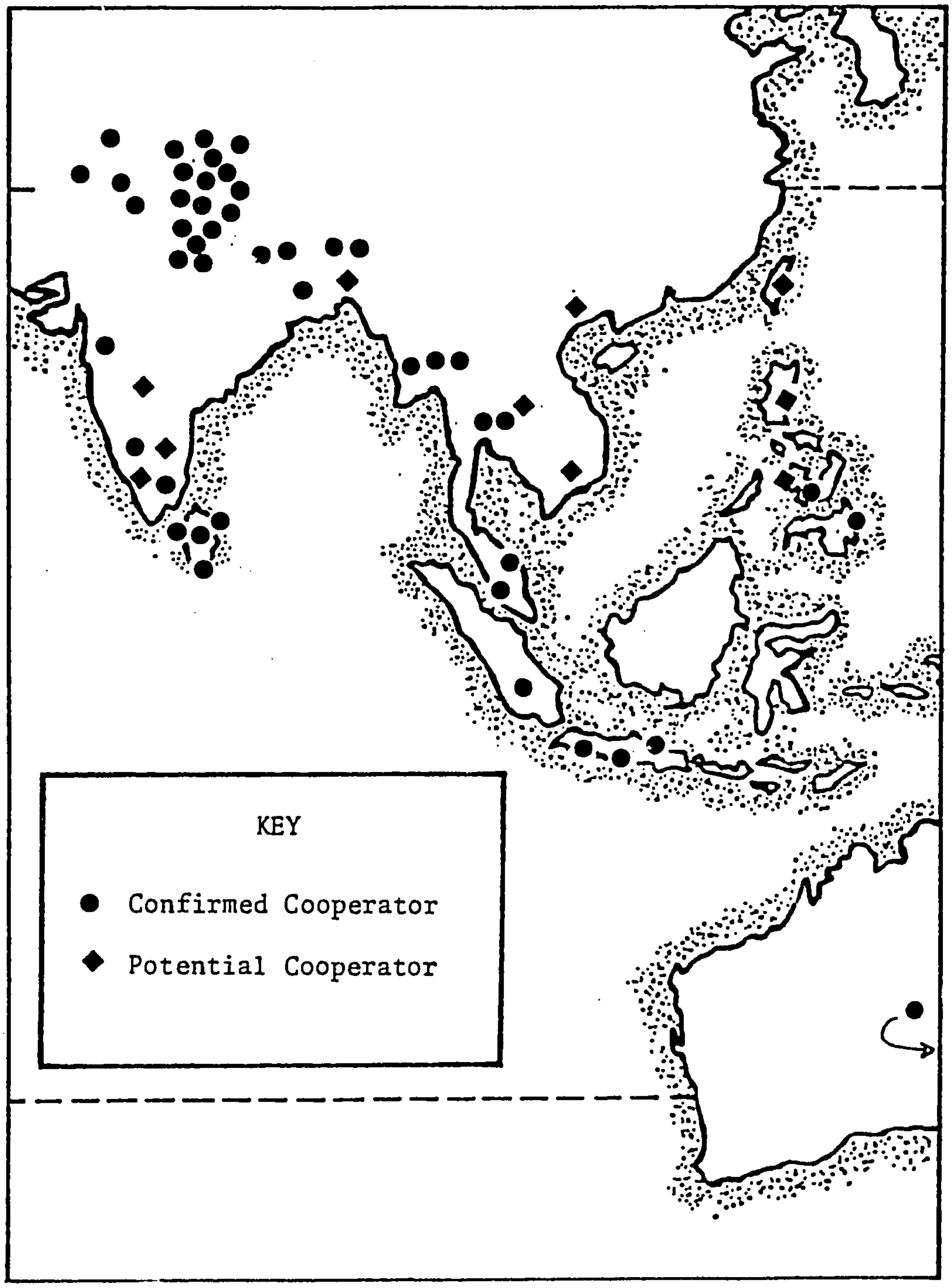


PARTICIPANTS IN INTERNATIONAL NETWORK OF LEGUME INOCULATION TRIALS

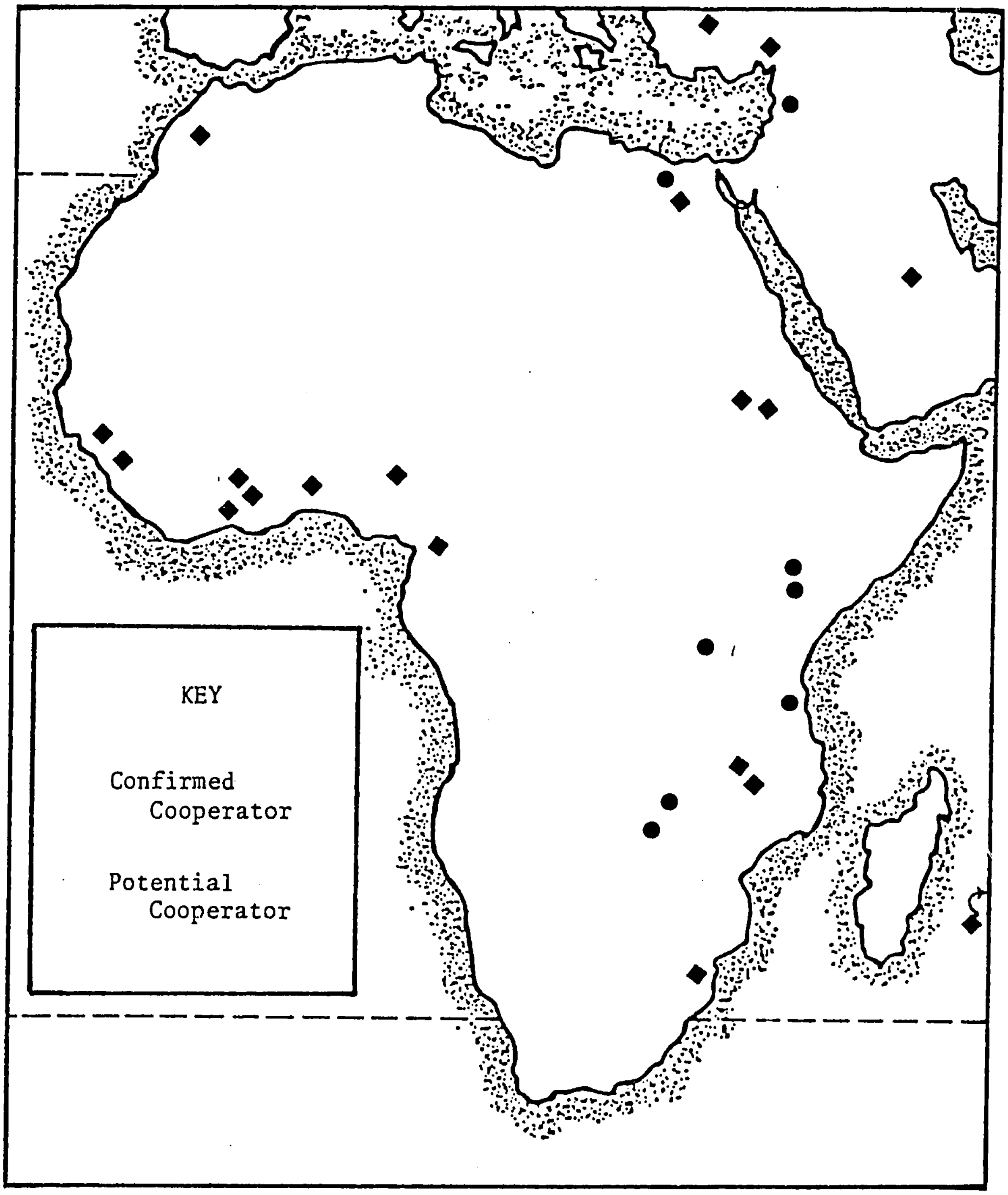


Distribution of Cooperators -- Americas Network





Distribution of Cooperators -- Asia Network



Distribution of Cooperators -- Africa Network

PARTICIPANTS

Country	Number of Cooperators
Brazil	5
Colombia	2
Costa Rica	1
Guyana	1
Mexico	4
Panama	2
Peru	3
Puerto Rico	2
Surinam	1
Trinidad and Tobago	1
United States	3
Venezuela	1
TOTAL:	26

POTENTIAL PARTICIPANTS

Country	Number of Cooperators
Brazil	2
Cuba	1
Dominican Republic	1
Peru	1
Venezuela	1
TOTAL:	6

LEGUMES

Legume	Number of Trials
Arachis hypogaea	2
Aeschynomene americana	1
Cajanus cajan	4
Centrosema pubescens	2
Cicer arietinum	2
Desmodium heterocarpum	1
Desmodium intortum	2
Desmodium ovalifolium	1
Glycine max	10
Leucaena leucocephala	11
Medicago sativa	2
Phaseolus vulgaris	8
Pueraria phaseoloides	2
Stylosanthes guianensis	6
Vigna mungo	1
Vigna radiata	1
Vigna unguiculata	2
TOTAL:	58

PARTICIPANTS

Country	Number of Cooperators
Bangladesh	2
India	39
Indonesia	2
Malaysia	2
Nepal	1
Pakistan	2
Philippines	1
Sri Lanka	1
Thailand	2
TOTAL:	52

COUNTRY-WIDE UNITS PARTICIPATING

Country	Number of Units
India	1
Indonesia	2
Malaysia	1
TOTAL:	4

POTENTIAL PARTICIPANTS

Country	Number of Cooperators
India	3
Nepal	1
Philippines	1
Sri Lanka	3
Thailand	1
TOTAL:	9

POTENTIAL COUNTRY-WIDE UNITS

Country	Number of Units
Bangladesh	1
India	2
Malaysia	1
Pakistan	1
Philippines	1
Sri Lanka	1
TOTAL:	7

LEGUMES

Legume	Number of Trials
Arachis hypogaea	4
Cajanus cajan	7
Calopogonium sp.	1
Centrosema pubescens	4
Cicer arietinum	11
Desmenthes sp.	2
Desmodium intortum	1
Glycine max	23
Lens culinaris	6
Leucaena leucocephala	6
Macroptilium atropurpureum	1
Phaseolus vulgaris	3
Pisum sativum	1
Pueraria phaseoloides	1
Stylosanthes guianensis	1
Vigna mungo	12
Vigna radiata	18
Vigna unguiculata	6
TOTAL:	108

PARTICIPANTS

Country	Number of Cooperators
Egypt	2
Gambia	1
Ghana	2
Kenya	2
Malawi	2
Nigeria	1
Rwanda	1
Saudi Arabia	1
Sudan	1
Syria	1
Tanzania	1
Turkey	1
Zambia	2
TOTAL:	18

POTENTIAL PARTICIPANTS

Country	Number of Cooperators
Cameroons	1
Liberia	1
Mauritius	1
Morocco	1
Senegal	1
TOTAL:	5

LEGUMES

Legume	Number of Trials
Arachis hypogaea	3
Cicer arietinum	4
Glycine max	11
Phaseolus vulgaris	8
Leucaena leucocephala	1
Lens culinaris	2
Medicago sativa	1
Vigna unguiculata	4
Vicia faba	2
TOTAL:	36

APPENDIX 5

PUBLICATIONS AND PRESENTATIONS

NifTAL Publications and Publications by NifTAL-Sponsored Personnel (FY 1979 - 1980)

Akyeampong, M. (1980) The effect of different strains of *Rhizobium* and phosphorus levels in mungbean (*Vigna radiata*) and soybean (*Glycine max*). M.S. Thesis, Univ. of Hi., Dept. of Agron./Soil Sci. 65 pp.

Berger, J. A., S. N. May, L. R. Berger, and B. B. Bohlool. (1979) Colorimetric enzyme-linked immunosorbent assay for the identification of strains of *Rhizobium* in culture and in the nodules of lentils. Appl. Envir. Microbiol. 37(3):642-646.

Bose, John II. (1980) The legume/*Rhizobium* symbiosis in tropical agriculture: a bibliographic update. NifTAL Project (Univ. of Hi. and USAID), 167 pp.

Cassman, K., A. S. Whitney, and R. L. Fox. (1980) Symbiotic nitrogen fixation by legumes requires extra phosphorus. Illustraed Concepts in Tropical Agriculture, Univ. of Hi., Coll. Trop. Ag. Hum. Res. 1 pg.

Cassman, K. G., A. S. Whitney, and K. R. Stockinger. (1980) Root growth and dry matter distribution of soybean as affected by phosphorus stress, nodulation, and nitrogen source. Crop Sci. 20(2):239-244.

Guevarra, A. B., Y. Kitamura, A. S. Whitney, and K. G. Cassman. (1980) A low-cost system for circulating nutrient solutions in pot studies. Crop Sci. (20(1):110-112.

Harris, S., Ed. (1980) *BNF BULLETIN* (Quarterly newsletter). U.S. Universities Consortium on Biological Nitrogen Fixation in the Tropics and NifTAL Project.

Harris, Susan C. (Ed.) (1979) Planning an International Network of Legume Inoculation Trials: the proceedings of a workshop held at Kahului, Maui, Hawaii, Jan. 15-19, 1979. NifTAL Project (Univ. of Hi. and USAID), 241 pp.

Keyser, H. H. and D. N. Munns. (1979) Tolerance of rhizobia to acidity, aluminum, and phosphate. Soil Sci. Soc. Am. Jour. 43(3):519-523.

Keyser, H. H., D. N. Munns, and J. S. Hohenberg. (1979) Acid tolerance of rhizobia in culture and in symbiosis with cowpea. Soil Sci. Soc. Am. Jour. 43(4):719-722.

May, S. N. (1979) Ecological studies on lentil rhizobia: competition and persistence in some tropical soils. M.S. Thesis, Univ. of Hi., Dept. of Microbiology. 54 pp.

NifTAL Project (1979) Annual Report. Univ. of Hi. and USAID. 59 pp.

Righetti, T. L. and D. N. Munns. (1980) Nodulation and nitrogen fixation in cliffrose (*Cowania mexicana*). Plant Physiol. 65:411.

Zaroug, M. G., and D. N. Munns. (1979) Nodulation and growth of *Lablab purpureus* in relation to *Rhizobium* strain, liming, and phosphorus. Plant and Soil, 53:329-339.

Zaroug, M. G. and D. N. Munns. (1979) Nodulation, nitrogen fixation, leaf area, and sugars content in *Lablab purpureus* as affected by sulfur nutrition. Plant and Soil. 53:319-328.

Zaroug, M. G. and D. N. Munns. (1980) Screening strains of *Rhizobium* for the tropical legumes *Clitoria ternatea* and *Vigna trilobata* in soils of different pH. Tropical Grasslands. 14(1):28-33.

*Exploiting the Legume/*Rhizobium *Symbiosis in Tropical Agriculture* (2nd edition, 1979)

432	- from Maui
200	- Ag. Expt. Stn.
35	- Oahu
300	- Hamilton Lib. Gifts & Exchange
967	- TOTAL

International Network of Legume Inoculation Trials Experiment A (1979)

285 - distributed

*The Legume/*Rhizobium *Symbiosis in Tropical Agriculture: A Bibliographic Update* (1980)

1st Printing - 1000 - Not yet distributed.

All persons who received 1979 Bibliography will receive Update automatically.

Planning a Network of Legume Inoculation Trials (1979)

569	- from Maui
50	- Oahu
300	- Hamilton Lib. Gifts & Exchange
919	- TOTAL

*Practical Exercises in Legume/*Rhizobium *Techonology* (Revised Ed., 1979)

15	- Nairobi course
50	- Porto Alegre MIRCEN courses
10	- Intern Trainees
15	- Administrators
90	- TOTAL

Spanish Edition is in preparation; translation complete.

BROCHURES

What is NifTAL (1979)	-	950
Training Opportunities (1979)	-	1000
An International Network (1980)	-	500
Training Opportunities (1980)	-	50
TOTAL (Approximate Distribution)	-	2500

NEWSLETTERS

BNF Bulletin

March, 1980 issue - 800 distributed
(generated 123 requests for subscriptions)
July, 1980 issue - (In press)

Notes from NifTAL (For Trainees)

December, 1979 issue - 65 distributed
May, 1980 issue - 78 distributed
143 TOTAL

Papers and Presentations Delivered at Meetings (FY 1979 - 1980)

Burton, J. Inoculant Production. FAO/UNEP Conference on Symbiotic Biological Nitrogen Fixation; Rome, Italy; Food and Agriculture Organization and United Nations Environment Program; June, 1980.

Cassman, K.* and D. N. Munns. Soil N availability and assessment of symbiotic N2 Fixation. Symposium on Enhancing Biological Production of Ammonia from Atmospheric Nitrogen and Soil Nitrate; Tahoe City, California; National Science Foundation and Univ. of California, Davis; June, 1980.

Davis, R. J. An International Network of Legume Inoculation Trials: The Asia Network. All India Coordinated Pulse Workshop; Kanpur, India; Indian Council of Agricultural Research (ICAR); April, 1980.

Davis, R. J.*, J. Halliday, and P. Somasegaran. An International Network of Legume Inoculation Trials. American Society of Microbiology Annual Meeting; Miami Beach, Florida; ASM; May, 1980.

Halliday, J. Training Programs in BNF. FAO/UNEP Conference on Symbiotic Biological Nitrogen Fixation; Rome, Italy; Food and Agriculture Organization and United Nations Environment Program; June, 1980.

McNeil, D. L.* and T. LaRue. N transport in soybeans; Hawaii Plant Physiology Meeting; Honolulu, Hawaii; Univ. of Hawaii; May, 1980.

Whitney, A. S. Chairman, Grazing Management of Tropical Pastures. Australian Conference on Tropical Pastures; Brisbane, Australia; CSIRO; May, 1980.

Paper was delivered by author indicated by asterisk.

APPENDIX 6

VISITORS

Chronological List of Visitors to the NifTAL Project (FY 1979 - 1980)

VISITOR	INSTITUTION
Chauncey Ching	Univ. of Nevada
Russell Yost	CTAHR/UHM*
Eli Sydney Lopes	Instituto Agronomico Brazil
Harold S. Matsumoto	UHM**
Tamotsu Sahara	UHM
Harold Tanaka	UHM
Tom Miura	Maui Community College
James L. Brewbaker	CTAHR/UHM
Rich Van Den Beldt	CTAHR/UHM
Roy H. Muramoto	USAID/UHM
Leona S. Craig	AID/DSB/IT
Lillian E. Mickens	AID/DSB/IT
Michael D. Benge	AID/DS/AGR (Agro-forestry)
Gordon Y. Tsuji	Benchmark Soils Proj./UHM
Patrick C. Ching	Benchmark Soils Proj./UHM
James Silva	BSP/CTAHR/UHM
Norbert H. Hale	Wailuku Armory
J. H. Okazaki	Wailuku Armory
Gus Higuchi	RCUH
A. Geoffery Norman	Univ. of Michigan
Frank Hiob	CTAHR/UHM
Jean S. King	State of Hawaii Lt. Gov.
Alan Hooper	Univ. of Minnesota
Hassan Moawad	Egyptian National Research Center
Burton Koch	Washington State Univ.
Fred Labasang	Haiku, Maui
Robert Traxler	U.S. House of Representatives
Robert Foster	U.S. House of Representatives
Jack	USDA (Fruit Fly Lab)
Edward L. Pulver	IITA, Nigeria
Carmel G. Young	Kula, Maui
Edward Bartholomew	Maui Community College
Harold H. Keyser	USDA/SEA/AR
Donald Munns	Univ. of California, Davis
Bruce S. Plasch	Honolulu, Hawaii
Shelley M. Mark	CTAHR/UHM
J. W. Newton	USDA Northern Lab
O. A. Denton	Nigerian National Hort. Research Ins
Nkere J. Usoroh	Nigerian National Hort. Research Ins
K. W. Bridges	CTAHR/UHM
Richard T. Imai	UHM
Alan T. Nakayama	UHM
Kenneth G. Cassman	IRI/Brazil
Marlowe Thome	Univ. of Illinois
Henry J. Baldwin	Haiku, Maui
Bjorn Solheim	Univ. of Tromsø/Norway
Philip Motooka	CTAHR/Hawaii County
Burton Smith	CTAHR/Hawaii County
	Maui Community College

Nobom Morishige	San Diego, Calif.
Kiyohara Shodal	Kahului, Maui
Isami Nakashiya	Wailuku, Maui
Lilia Brewbaker	Kailua, Hawaii
Bruce Koppel	East-West Center
Saleem Ahmed	East-West Center
Tom Hill	BSP/Molokai
L. G. Jefts	Hawaii
Wayne Hiets	Hi. Sugar Planters' Assn.
R. P. Bosshart	Hi. Sugar Planters' Assn.
Alan K. Hiyashi	Puunene, Maui
Faik Al-Nashi	Baghdad, Iraq
Fariq Al-Khamis	Baghdad, Iraq
Ross Rainbird	Univ. of Western Australia
Janice Rainbird	Perth, Australia
James Brewbaker	CTAHR/UHM
Glenn Ito	CTAHR/UHM
Stan Pedersen	CTAHR/UHM
M. Sudjadi	Indonesia Soil Research Inst.
Perfecto R. Vicente	BSP/Philippines
Martin E. Raymundo	PCARR/Philippines
Patrick Ching	BSP/UHM
Jerry L. McIntosh	CRIA/IRRI/Indonesia
R. George Manuelpillai	BSP/Indonesia
James L. Walker	AID/DSB/OA
K. N. Satyapal	UNDP/New York
Edwin Mersins	Honolulu, Hi.
Morgan Childs	Killington, VT
J. M. Poehlman	Univ. of Missouri

*College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa

**University of Hawaii at Manoa

APPENDIX 7

NifTAL GROUNDS

HAMAKUAPOKO, MAUI

NIFTAL GROUNDS
HAMAKUAPOKO, MAUI, HAWAII