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ANNUAL REPORT

TO

U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT

JULY 1979 - JUNE 1980

"BIOLOGICAL NITROGEN FIXATION FOR IMPROVED FOOD PRODUCTION IN THE TROPICS"

GRANT NO : AID/DSAN-G-0100

PRINCIPLE INVESTIGATOR: B. Ben Bohlool

SUBMITTED BY : College of Tropical Agriculture
& Human Resources
University of Hawaii
Honolulu, Hawaii

Introduction

In 1979, the Agency for International Development (AID) awarded a 3-year institution-strengthening grant to the University of Hawaii (UH) for implementation of studies on utilization of biological nitrogen fixation (BNF) in the tropics. The activities were to be coordinated with those of three other universities (Cornell, North Carolina State, and Puerto Rico) from the consortium on Soils of the Tropics.

The UH commitment encompasses several aspects aimed at implementation of BNF knowledge in less-developed countries (LDC) in the tropics. The activities are outlined in Table 1, along with UH staff members directly involved.

I. Expanded Knowledge Base

This component of the grant is concerned with studies that would enhance our understanding of two areas in BNF: Rhizobium ecology in saline tropical soils; and the agronomic potential of *Azolla* in tropical farming systems. The relationships of these two areas of investigation to other activities at the UH are shown in Figure 1. The details are given below:

A. *Rhizobium* in saline tropical soils:

Saline soil conditions may affect the legume--*Rhizobium* symbiosis in a variety of ways. Salinity and its related effects could (1) limit survival of inoculum rhizobia on seeds, (2) retard growth and proliferation of introduced rhizobia in the rhizosphere, (3) adversely affect the ability of the desired rhizobia to nodulate, (4) restrict the nitrogen-fixing function of the nodule, and (5) prevent long-term persistence of desired inoculum strains; or any combination of these affects.

It is, therefore, important to understand the effect of salinity stress at various stages of the process. The focus of our studies in the first year of this grant has been the study of the tolerance of Rhizobium isolates to salinity in artificial media and sterilized soils.

Twenty-eight Rhizobium isolates from diverse legumes growing on beaches and salt-affected agricultural land have been tested for their growth re[illegible] increasing concentrations of salt and their persistence in gamma-[illegible]d salinized soils. In general, we have found that rhizobia isolated from saline soils are more resistant to salinity affects than those from non-saline areas. However, all strains tested show a remarkable degree of tolerance to salinity, far beyond the limits of most agricultural crops.

B. Agronomic potential of Azolla in tropical farming systems:

Research on biological nitrogen fixation by Azolla at the University of Hawaii is funded by AID 211(d) and USDA section 406. Several different lines of research are being conducted or are planned using funds from the two sources.

Mr. Thomas Lumpkin, whose salary is paid by the 406 project, traveled throughout the world to collect the known species of Azolla. These species were sent to the People's Republic of China where Tom is currently studying the contribution by the different Azolla species to the nitrogen economy of rice. Most aspects of the evaluation of the agronomic potential of the species are being supported with 211(d) funds. Pot studies are being made of dry matter production, persistence, and acetylene reduction rates by the different species. Data have been collected throughout most of one year to evaluate the effects of seasonal variation in climate on Azolla.

Under the climatic conditions at Hangzhou, Zhejing, People's Republic of China, growth and nitrogen fixation of all Azolla species dropped off sharply at temperatures below 15° C. Nitrogen content was 2.5 to 3.5 % in the winter months but increased to 3 to 5 % by April. Doubling times for Azolla biomass was about 10 days in April but decreased to 3 to 6 days by June. In May, as mean temperature increased, nitrogen fixation rates for all Azolla species and strains were similar. By mid-June, the tropical and subtropical species A. niolotica and A. microphylla were fixing nitrogen at two to five times the rate of the temperate species. At temperatures above 40° C, only the tropical varieties continued to fix nitrogen.

Six strains and species of Azolla have been grown with rice in 24 m^2 field plots and rice yields were taken to evaluate the ability of Azolla to supply nitrogen to rice. The cool season A. filiculoides produced the greatest biomass and supplied the most N to the rice crop. Rice yields were high even with no added nitrogen and differences between treatments were small suggesting that there was significant residual N in the paddies.

A second field experiment was installed in midsummer to evaluate the nitrogen contribution of the tropical species of Azolla to rice. Just prior to spreading of the Azolla, water temperatures in the nursery rose to 45° C and significant quantities of the cool season species were lost. However, the tropical species survived and were used in the field experiment. The results conclusively demonstrate that the Azolla species can be grouped on the basis of their response to temperature.

Research on the rate of decomposition and release of nitrogen from Azolla incorporated into paddy soil is being conducted by Barbara Bellows, a graduate student supported by section 406 funds. Data are being collected on

rice yields, nitrogen content and changes in available NH_4^+ for flooded pots and NH_4^+ and NO_3^- for alternating wet-dry conditions.

As a part of the study of rate of N release from Azolla, three species of the fern will be labeled with ^{15}N. Labeled Azolla or labeled NH_4NO_3 will be added to pots and the relative rates of uptake of ^{15}N from the fern and the chemical fertilizer will be determined. The ^{15}N studies will be conducted using 211(d) funds.

Dr. Ramon de la Pena, an agronomist on the Island of Kauai, has initiated studies of Azolla filiculoides as a green manure crop. Later work will evaluate the N contribution by Azolla to taro (Colocasia esculentum), an important starch crop in Hawaii and the tropics.

Recently, Mr. Kofi Debrah from Ghana, a master of science candidate in Agronomy and Soil Science, was provided with a research assistantship funded by the 211(d) grant. Mr. Debrah will study the relationship between light, Azolla growth and nitrogen content.

The 211(d) project has also been used to pay travel expenses for Mr. Lumpkin to national meetings on Azolla in China. Much of the Chinese literature on Azolla has been collected and sent to the library at the University of Hawaii. In addition, publication of the following article was made possible by the AID 211(d) grant. Lumpkin, T.A. and D. L. Plucknett. 1980. Azolla: botany, physiology, and use as a green manure. Economic Botany 34:111-153.

II. Education and training:

This activity of the project is an integral part of the "Expanded Knowledge Base" and the "Information" components of the program. Graduate students and postdoctoral trainees will benefit from the research and educa-

tional activities and, in turn, contribute to the over all objectives of the grant. Qualified candidates from LDC's have been encouraged to apply. In the first year, one graduate student and one project associate (postdoctoral) were funded by the project.

III. Information capacity:

A. The State-of-the-Arts in green manuring and cover cropping practices in the tropics:

Objectives of the study:

1. Summarize general concepts of the use of green manures and cover crops (GM/CC) which have resulted from farmer practice and research experience in the tropics.
2. Identify aspects of the practice of GM/CC which are inadequately understood and identify constraining information gaps.
3. Prepare a publication which identifies important factors in GM/CC practies and which assists agriculturists and extension personnel in choosing practices which may be appropriate for their situations.

Progress to date:

Summary and preparation of the bibliography is progressing with approximately 900 citations in the card file, about 300 of which have been keyworded and placed in a computer file for retrieval by subject matter, author, region, and article type. A questionnaire was prepared and distributed in January, 1980. The data from 50 respondentshas provided considerable detail on the GM/CC practice in their regions.

The questionnaire results indicate that green

manuring and cover cropping are not highly correlated.
For example, green manuring appears to be far more common in Asia than in Africa, Tropical America, and Oceania where the practice is rare. Cover cropping was somewhat more frequently practiced in Oceania and Africa than in Tropical America. Travel sites are being selected and communications prepared. The earliest travel should be late 1980/ early '81.

Work plan for 1980-1981:

1. Continue developing bibliography.
2. Formulate and adopt outline for the major summary paper.
3. Explore feasibility of a green manure species-climate grouping system.
4. Visit centers of GM/CC research and practice.
5. Prepare an interim summary paper of results.

B. Completion of SOTA's from previous grant:

The current grant has aided in the competition of three SOTA's commissioned under a previous 211(d) grant:

1. "Evaluation of N_2-Fixation by Bacteria in Association with the Roots of Tropical Grasses", Van Berkum, P. and Bohlool, B. B. Published as a review article in Microbiological Reviews 44(3) 1980, 491-517.
2. "VA Mycorrhiza in Tropical Agriculture", Mosse, B. To be published as a UHAES-technical Bull. in early 1981.
3. "Contribution of Centrosema, Desmodium and Stylosanthese to BNF in Tropical Pastures", edited by Burt, R., Walker, J. and Rotar, P. P. To be published as a book in 1981.

IV and V. Advisory Capacity/Linkages

The activities within these categories have been integrated together in order to provide the cohesive structure needed for establishing strong linkages with LDC organizations to, first of all, work more effectively on the research and training aspects of this project while, at the same time, respond to LDC requests for technical assistance.

A. Advisory and linkage activities regarding BNF in Leucaena in Thailand:

In the first year of this grant, UH responded to a request from the Royal Thai Forest Department, through USAID/Bangkok, for advisory services in the production of Leucaena. A three-man team visited Thailand during February 26 - March 8, 1980, to consult on symbiotic nutrition of Leucaena introduced to stress soils. The 211(d) grant provided travel and/or support for Ta Wei Hu (Taiwan Forestry Research Institute), James Brewbaker (UH, Horticulture Dept.), and Jake Halliday (UH, Agronomy and Soils NifTAL Project) to visit Bangkok and other regions in Thailand to provide the requested assistance.

A series of collaborative projects was initiated by the team, notably with the Royal Thai Forest Department personnel, on Leucaena silviculture, density, provenance and rhizobial inoculation.

Follow Up: The short rainy season, low total precipitation and sporadic rainfall distribution impose the greatest constraint to reforestation of denuded areas of North and Northeast Thailand. A collaborative assistance project on establishment of fast-growing nitrogen fixing trees in the short rainfed season was tentatively agreed upon. A project proposal was endorsed verbally by AID/Bangkok, RTFD Land Management

and Silviculture Divisions, Thai Department of Agriculture (Plant Pathology Division) and the University of Hawaii. A research proposal on "Appropriate technology for Legume Tree Propogation" was submitted to USDA SEA/CR to extend the cooperative aspects of the studies initiated during this trip.

B. Rhizobium/Salinity aspect:

We have initiated moves to establish linkages with scientists in Egypt. The focus of our collaboration is the soil microbiology section of the Egyptian National Research Center (NRC). Our aim, however, is to utilize this connection to carry out collaborative experiments at the Salinity Laboratories in Alexandria. We are currently negotiating with Dr. Hassan Moawad Abdel Al of NRC to spend from 9-12 months at the UH as a visiting professor.

C. Azolla utilization in Southeast Asia and People's Republic of China:

Tom Lumpkin's stay in China has been used as a focal point for organizing a workshop on Azolla technology aimed for 1981/82 in Thailand. Several scientists in tropical LDC's have been contacted regarding this workshop, and also for cooperating on Azolla research.

Summary and Conclusions

The UH is on target for the projected objectives stated in Table III, Log Frame, and Table IV, Milestone Events, of the project proposal. This has been helped partly by the momentum that was gained in BNF during the previous 211-d grant activities, and partly by the fact that other projects at the UH inter-connect with the project in achievement of our objectives.

In the "Expanded Knowledge Base" section we have isolated several strains of Rhizobium and characterized their salt tolerance. We find rhizobia in general to be extremely tolerant of salt. We do not, therefore, expect rhizobia to be the limiting factor in the establishment of legumes in saline soils.

All the known species of Azolla have been collected and their relative growth potential at different temperatures studied. So far, we can conclude that Azolla can be divided into "tropical" and "temperate" types on the basis of their response to temperature. Several of these are currently being field-tested in China.

Three BNF-related SOTA's from a previous assignment have been completed with the aid of 211-d funds. The current SOTA commitment on "Green Manures and Cover Crops" is far ahead of its schedule. Literature on the subject has been compiled and is being continually updated. A questionnaire on GM/CC practices in tropical LDC was distributed to scientists and agriculturalists in 33 countries. The analysis of the results indicate a disparity in the practices of GM and CC in different countries in the tropics.

In this first year of our activities, we have initiated linkages with Thailand, Egypt and People's Republic of China. We intend to strengthen these ties by seeking other sources of funds for research and

training related to the needs of these countries.

Problems

(1) *Rhizobium* research has so far been limited to studies in the laboratory. We will initiate field studies in the second year of the grant.

(2) Firm and long-lasting linkages with established institutions are difficult to make and maintain under a short-duration grant with limited financial allotment for this activity. The budget will be redirected to provide a larger share for Advisory/Linkage and LDC-Training components.

Table 1. OUTLINE OF THE PERTINENT DETAILS OF THE 211-d PROJECT

TITLE: Biological Nitrogen Fixation for Improved Food Production in the Tropics

STARTING DATE: July 1, 1979

I. Expanded Knowledge Base (Research).

A. Rhizobium Ecology: Inoculant survival in tropical soils. Salinity as a stress factor for BNF.

Personnel Involved: Bohlool (lead scientist)
El-Swaify
Habte

B. Agronomic Potential of Azolla in Tropical Farming Systems:

Personnel Involved: Bartholomew (lead scientist)
Habte
de la Pena (Kauai)
Lumpkin

II. Education and Training.

Includes 1 graduate student and 1 postdoctoral.

III. Information Capacity:

The preparation of a major State-of-the-Art (SOTA) document on green manures and cover crops in tropical cropping systems.

Personnel Involved: Kanehiro (lead scientist)
Yost (project associate, 50%)

IV. Advisory Capacity:

To utilize UH expertise from a variety of disciplines related to BNF to respond to requests from less developed countries for technical assistance.

Personnel Involved: All UH faculty with expertise in microbiology and/or tropical soils (Bohlool, lead scientist).

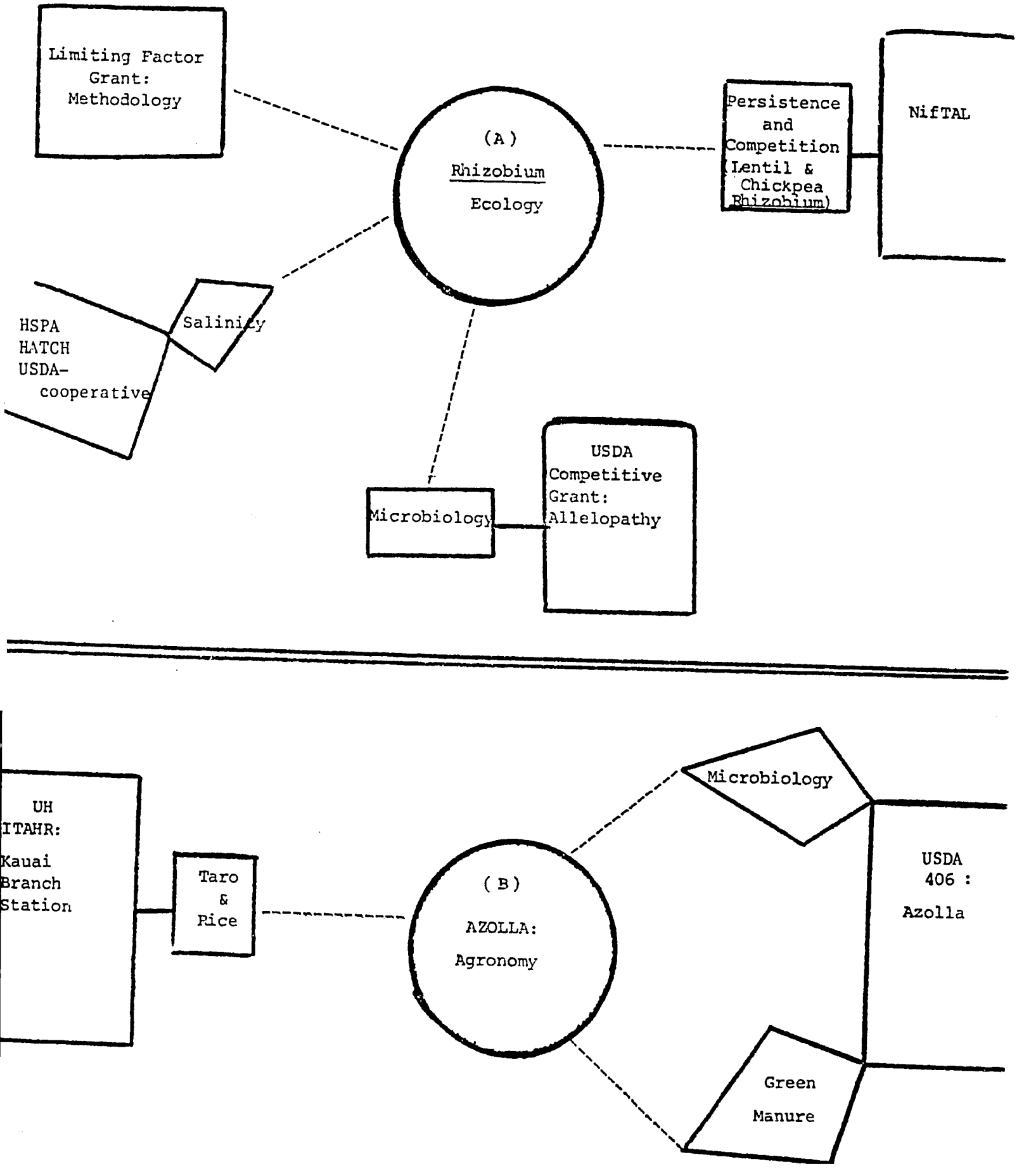
V. Linkages and Networks:

To establish strong linkages with domestic, multilateral and less developed country organizations to work more effectively on the research and training aspects of this project.

Personnel Involved: All UH faculty involved in BNF-related activities (Halliday, lead scientist).

Figure 1

RELATION OF 211-d RESEARCH TO OTHER UH PROGRAMS



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University of Hawaii at Manoa

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Cable Address: UNIHAW

October 30, 1980

Dr. L. R. Frederick
Office of Agriculture
Bureau for Development Support
Agency for International Development
Department of State
Washington D. C. 20523

Dear Lloyd,

Enclosed please find the original and four (4) copies of the UH Report of Annual Progress under the 211-d grant.

Look forward to seeing you in Raleigh, if not in D. C.

Sincerely,

B. Ben Bohlool
Associate Professor

BBB:jkw

Enclosure

xc: N. P. Kefford
P. P. Rotar

AN EQUAL OPPORTUNITY EMPLOYER

SUMMARY REPORT
1980

211-d Grant: AID/DSAN-G-0100 - University of Hawaii, P.I. Bohlool, B.B.

TITLE: "Biological Nitrogen Fixation for Improved Food Production in the Tropics"

SOTA reports

1. Berkum, van P. and Bohlool, B. B. 1980. Evaluation of Nitrogen Fixation by Bacteria Associated with the Roots of Tropical Grasses. Microbiological Reviews. 44: 491-517.

2. Mosse, B. 1981. VA Mycorrhiza in Tropical Agriculture. UHAES Technical Bulletin.

3. Burt, R., Walker, J. and Rotar, P. (in preparation). Contribution of Centrosema, Desmodium and Stylosanthere to BNF in Tropical Pastures. To be published as a book.

Other Publications

1. Bohlool, B. B. and Schmidt, E. L. 1980. The Immunofluorescence Approach in Microbial Ecology. Advances in Microbial Ecology. 4: 203-241.

2. Lumpkin, T. A. and Plucknett, D. L. 1980. Azolla: Botany, Physiology and Use as a Green Manure. Economic Botany. 34: 111-153.

3. Berger, J., May, S., Berger, L. and Bohlool, B. 1979. Colorimetric Enzyme-Linked Immunosorbent Assay for Identification of Strains of Rhizobium in Culture and in the Nodules of Lentils. Applied Environmental Microbiology. 37: 642-646.

4. Kinglsey, M. and Bohlool, B. Release of Rhizobium from Tropical Soils and Their Recovery for Immunofluorescence Enumeration. (Submitted to Appl. Environ. Microbiol.)

Theses Completed

1. May, S. N.
Ecological Studies on Lentil Rhizobia: Competition and Persistence in Some Tropical Soils.
M. S. Thesis, University of Hawaii, 1979.

2. Kingsley, M. T.
Release and Recovery of Rhizobium from Tropical Soils for Enumeration by Immunofluorescence.
M. S. Thesis, University of Hawaii, 1980.

Graduate Students

1. Paul Singleton*
2. Debra Kofi*
3. Michael Sadowsky *
4. Thomas Lumpkin**
5. Barbara Bellows**
6. Renee Kosslak

Visiting Scientists

1. Barbara Mosse - 1978*
2. Peter van Berkum - 1979*
3. Hassan Moawad Abdel Al - 1980-1981*

Project Associates

Russell Yort*

*Supported by AID-211-d

**Supported by USDA 406 and AID-211-d