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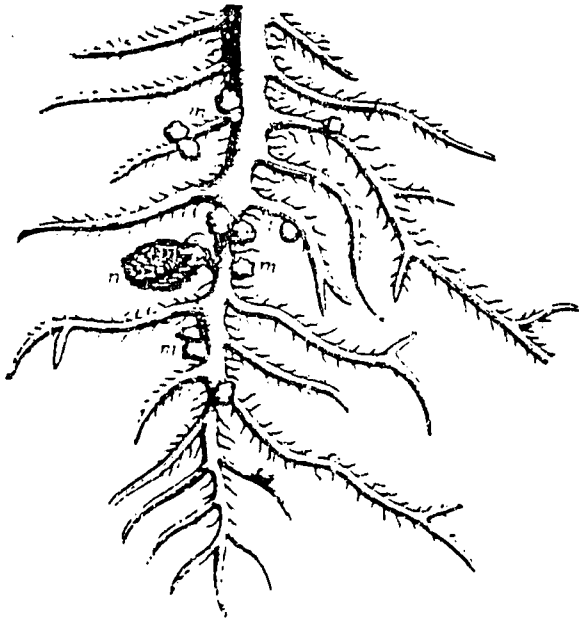
BIOLOGICAL NITROGEN FIXATION FOR FOOD PRODUCTION IN THE TROPICS

FINAL REPORT

AGENCY FOR INTERNATIONAL DEVELOPMENT

GRANT TO STRENGTHEN THE INSTITUTIONAL RESPONSE CAPABILITIES OF THE UNIVERSITY OF PUERTO RICO, CONTRACT AID/DSAN-G-0101



Nodules of *Vicia faba*

(Malpighi, 1679)

BIOLOGICAL NITROGEN FIXATION LABORATORY (BNF)

DEPARTMENT OF AGRONOMY AND SOILS

COLLEGE OF AGRICULTURAL SCIENCES

UNIVERSITY OF PUERTO RICO AT MAYAGUEZ

1979-1985

BIOLOGICAL NITROGEN FIXATION FOR FOOD PRODUCTION IN THE TROPICS

BNF

FINAL REPORT

USAID CONTRACT DSAN-G-0101

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Dr. Eduardo C. Schröder, Principal Investigator

Department of Agronomy and Soils

College of Agricultural Sciences

UNIVERSITY OF PUERTO RICO

MAYAGUEZ CAMPUS

MAYAGUEZ, PUERTO RICO

STATISTICAL SUMMARY

TITLE	Biological Nitrogen Fixation for Food Production in the Tropics (BNF Project)
CONTRACT NUMBER	AID/DSAN-G-0101
PRINCIPAL INVESTIGATOR	Dr. Eduardo C. Schröder
ADDRESS	BNF Laboratory Department of Agronomy and Soils College of Agricultural Sciences University of Puerto Rico Mayaguez, Puerto Rico 00708 U.S.A.
CONTRACTOR	University of Puerto Rico Mayaguez Campus Mayaguez, Puerto Rico 00708
CONTRACT PERIOD	January 1, 1979 - December 31, 1985
REPORTING PERIOD	January 1, 1979 - December 31, 1985
USAID FUNDS TO DATE	$360,000
TOTAL USAID FUNDING	$360,000
PROJECT OFFICE	S & T/AGR/RNR
PROJECT MONITOR	Dr. Lloyd R. Frederick

NARRATIVE SUMMARY

During the grant period the College of Agriculture of the University of Puerto Rico has significantly expanded its knowledge base relative to Biological Nitrogen Fixation, and in particular on legume symbiotic systems. Research with tropical grain legumes (pigeonpeas, beans and soybeans), emphasized the importance of strain selection for effectiveness and ability to compete with adapted strains. Results from work on forage legumes (leucaena and naturalized species) show the potential of pasture legumes to provide protein rich diets for grazing animals. Information obtained from the nodulation status of some native trees and shrubs points to the fact that our knowledge on tropical Rhizobium is still very limited.

The grant had a marked effect on the curriculum of the Department of Agronomy and Soils. The undergraduate course on Soil Microbiology is now taught yearly, and a new graduate course on "Advances in biological Nitrogen Fixation" was developed during the grant period. Teaching and research conditions improved considerably due to improvements in classroom and laboratory facilities, library acquisitions and audiovisual equipment purchase. Other training activities involved the participation in the International Training Course in Rhizobium Technology organized by NifTAL and held in Mexico (1981), and in the Regional Training Course on Symbiosis between Rhizobium and Tropical Legumes of Agricultural Importance in Costa Rica (1983). The UPR-BNF Project co-sponsored the First Caribbean BNF Seminar and Training course held in the Dominican Republic in August, 1983 and helped organize the Caribbean Rhizobium Group (CRG), a regional group of the Latin American Rhizobium Association (ALAR).

In November of 1982, the University of Puerto Rico BNF Project and Title XII Strengthening Grant Program, sponsored and held an International Workshop on Practical Applications of Azolla for Rice Production. In the workshop, more than 50 participants from 12 different countries were able to discuss basic and applied aspects of Azolla and rice production, and participate in a field trip to Puerto Rico rice growing area.

An important accomplishment of the grant was the information output. Two SOTA books, one book on the Azolla Workshop Proceedings, three meetings abstracts, 10 papers (published or in press) and three M.S. theses were generated by the grant. Furthermore, BNF teaching materials were prepared and distributed; data for a Rhizobium strain catalog and legume germplasm collection were gathered and stored in magnetic media for microcomputer access.

Linkage and cooperative activities included countries such as Dominican Republic, Mexico, Guatemala, Perú, Brazil, Costa Rica, Haití and other in the Caribbean region. Therefore, an informal network of scientists interested in Rhizobium was developed, and the information disseminated through the publication of the CRG Circular. The University of Puerto Rico research capacity developed during the grant is being utilized through a research program in the Dominican Republic and participation in the USDA S-170 Regional Project.

TABLE OF CONTENTS

SECTION	Page
Title	i
Statistical summary	ii
Narrative summary	iii
Table of contents	1
Preface	2
Acknowledgements	2
Introduction	3
Research projects	3
Education and training activities	9
State of the Art Documents	11
Azolla and rice workshop	11
Consultancy, linkages and Cooperatie Activities	12
Other Activities	15
Grant impact and future plans	16

Appendices

I.	Meetings and Trips	17
II.	BNF Publications	20
III.	Project Personnel	23
IV.	Visitors list	24
V.	Expenditures report	26

PREFACE

In 1979, the United States Agency for International Development (USAID) approved an institutional strengthening grant to the University of Puerto Rico (UPR) to study Biological Nitrogen Fixation (BNF) for Food Production in the Tropics. This contract, as well as its predecessor (Tropical Soils), have helped the College of Agriculture of the University of Puerto Rico, Mayaguez Campus, to develop and improve a Soil Microbiology Laboratory, with emphasis on Biological Nitrogen Fixation research capabilities. The activities were to be coordinated with those of three other universities (Cornell, North Carolina State University, and University of Hawaii), from the Consortium on Soils of the Tropics.

It has been a privilege for the UPR BNF Project to participate in this program aimed toward full use of biologically fixed nitrogen in the tropical cropping systems of less-developed countries (LDC's).

ACKNOWLEDGEMENTS

The University of Puerto Rico acknowledges the continuing support by USAID for the BNF Laboratory, initially through grant AID/csd-2857 and under AID/DSAN-G-0101 until December 31, 1985. The budget provided by USAID permitted the redesign of the laboratory facilities to separate sterile area from general laboratory and autoclaving and cleaning (preparatory room). It was also instrumental in obtaining new funding for research activities.

The BNF Project also recognizes the strong suport received from the scientists and personnel of the Agricultural Experiment Station where research was conducted, and the cooperation of the Research Department of the Commonwealth Department of Natural Resources to conduct a survey of the legumes nodulation on its natural sites.

Thanks are also due to the individual farmers and agricultural enterprises that cooperated in one way or another to achieve our objectives.

INTRODUCTION

In 1979, the U.S. Agency for International Development (AID) aproved an institutional strengthening grant to the University of Puerto Rico (UPR, Mayaguez Campus) to study Biological Nitrogen Fixation (BNF) for Food Production in the Tropics. This contract, as well as its predecessor (Tropical Soils), have helped the College of Agriculture of the University of Puerto Rico, Mayaguez Campus, to develop and improve facilities for a Soil Microbiology Laboratory, with emphasis on Biological Nitrogen Fixation. Building improvement and the acquisition of new equipment for the laboratory have resulted in the most modern and the best facilities available in the Caribbean area for Biological Nitrogen Fixation studies.

The objective of the BNF grants from USAID to the four universities of the Consortium (Cornell, Hawaii, North Carolina and Puerto Rico), was to improve the institutional response capacity in the area of Biological Nitrogen Fixation.

The objectives of the University of Puerto Rico BNF Project are better considered under the folowing three types of outputs:

1. Research to explit BNF for crop production
2. Training personnel from LDC's and
3. Advisory activities

RESEARCH PROJECTS

1. Leucaena leucocephala inoculation trials

In coordination with NifTAL (Hawaii), experiments following the basic design of the International Network of Legume Inoculation Trials (INLIT) were set up. The objective of this project was to determine whether there is a need to inoculate Leucaena leucocephala under Puerto Rico soil conditions. L. leucocephala cultivar K-8 was established on July 30, 1980 at the Isabela Experimental Station on a pH 6.6 oxisol. The three basic treatments included were:

a. inoculated with Rhizobium (TAL 82, TAL 582 and TAL 1145)
b. not inoculated
c. not inoculated and fertilized with N (urea)

All treatments were included at the two fertility levels: treatments indicated by F received no additional fertilizer; treatments indicated by M received additional phosphorus and potassium.

Two weeks after planting, estimates of emergence indicated that N-fertilized treatments had significantly lower germination.

Nine weeks after planting, plants were sampled and nodules counted and identified serologically. The number of nodules found on the N- fertilized treatment were lower than at the other treatments. Non inoculated controls did not differ from inoculated plants in the number of nodules indicating that adequate numbers of native Rhizobium specific for Leucana were present in the soil. Serological analysis showed that 98% of the nodules reacted with the antiserum belonging to the inoculant strains, of which 72% of the nodules tested were formed by TAL 582 (CB 81).

Leucaena was harvested five times and dry matter yield/ha calculated. Inoculated treatments gave significantly higher yields, but P and K fertilization did not increase yields. N contents were not affected by experimental treatments. The high yields obtained (up to 16 tons dry matter/ha) indicate the need for continued research on Leucaena as a potential forage for cattle or biomass production in Puerto Rico.

2. Pigeonpea inoculation trials

The objective of these experiments was to determine, under field conditions, whether the yield of pigeonpeas could be increased by inoculating with selected strains of Rhizobium. Two experiments were planted at Isabela and Fortuna substations. The basic treatment included:

a. seeds inoculated with a specific strain of Rhizobium (8 treatments

b. non-inoculated seeds

c. non-inoculated seeds plus nitrogen (urea)

The 10 treatments were replicated four times. Since pigeonpea does not respond to fertilization in Puerto Rico, neither P or K were applied. Plots consisted of 4 rows arranged in a 2.4 m x 7.5 m area. Cultivar 2B-Bushy (determined) was sown.

The experiment at Fortuna Experimental Station gave very erratic results due to soil factors (herbicides or high N content) and damage by insects. Plants examined for nodulation lacked nodules and Rhizobium counts indicated that population was below the minimum to be detected.

Plants at Isabela Experimental Station were sampled 30 days after planting (DAP) and plant height was measured. Sampling for nodule count, weight and identification was done 45 DAP. At harvest, two central rows of each plot were harvested and green pod yield measured. Plant samples were harvested, dried, ground and analyzed for N content. There were small differences among treatments in plant height, nodule weight and nodule numbers. Strains 3223 and IHP 100 (India) produced significant higher yields but the N fertilization treatment had the maximum total N (Kjeldahl). No overall superior treatment was obvious. This can be explained with the results from serological analysis, since most nodules were formed by non-inoculant strains. Only strain IHP 155 (India) formed over 30% of the nodules when introduced in the inoculant. These results indicate the need to keep selecting Rhizobium strains able to fix N and compete with local strains, and to determine whether a larger number of bacteria at inoculum can increase pigeonpea yields.

3. Evaluation of inoculation methods for pigeonpeas (Cajanus cajan)

The objective of this experiment was to compare the nodulation and yield response of pigeonpea (Cajanus cajan) to inoculation with Rhizobium spp. utilizing different inoculation methods.

Previous experiments (see above) indicated that pigeonpeas does not respond to seed inoculation under Puerto Rican conditions. It is possible that unfavorable conditions prevent nodulation by the inoculant strain. Therefore, other inoculation methods were tested, aimed at:

a) increasing the number of nodules formed by the strain used in the inoculant, and

b) testing the competitiveness of introduced strains with indigenous populations of Rhizobium.

A field experiment was planted at the Fortuna substation (Mollisol) on September 25, 1981. Pigeonpea line 147 was sown. Plots consisted of 4 rows arranged in a 2.4 x 7.5 m area. Treatments were: 1) control not inoculated; 2) not inoculated with N fertilizer; 3) seed inoculation

(arabic gum slurry); 4) seed inoculation (pelleting with rock phosphate); 5) granular inoculant (soil applied) and 6) liquid soil inoculant. Plots were hand sown and precautions to avoid contamination were stressed.

Plants were sampled for nodulation and strain identification 35 DAP. Green pods were harvested on January 12, 1982, using the 2 center rows. Statistical analysis of nodule number/plant showed no significant differences among inoculation methods, but N fertilization gave the largest number of nodules/plant. Commercial strain 176A22 str resistant was utilized to prepare the inoculants. Strain identification of the marked strain revealed an extremely low percentage of nodules (0.9%) occupied by the inoculant strain. It has been reported that strain 176A22 is a very poor competitor, and selection of competitive strains should be carried out before comparing inoculation methods or inoculation rates. Analysis of variance (ANOVA) of the green pod yields showed no difference among treatments means.

4. Effect of mulching on Phaseolus vulgaris nitrogen fixation

The objective of this experiment was to evaluate the effects of different kinds of mulches on the symbiotic relationship of *Rhizobium phaseoli* and *Phaseolus vulgaris.*

A field experiment was established at the Adjuntas substation (Limaní) in January, 1982. Treatments applied to each plot included: a) coffee pulp; b) coffee straw; c) leucaena leaves; d) black plastic; e) "aluminum" plastic, f) bagasse, and g) control. Plots (2.4m x 5.0 m) were arranged in a randomized complete block design with 4 replications. All seeds except control treatments were inoculated with a commercial culture of *Rhizobium phaseoli*.

During the growth period, samples were taken to evaluate root nodulation (number and weight) and dry weight of plant top. Soil temperature and water content at 2 depths were followed by daily measurements thorugh soil probs. At harvest time, yield of grain and dry weight of serial parts were determined. Analysis of variance of the number of nodules showed significant differences among the treatments. Several inoculated treatments had a larger number of nodules than the uninoculated control, indicating some positive response to inoculation. Coffee pulp mulch treatment showed the highest number of nodules/plant, as well as the highest nodule weight. However, the fact that N fertilization produced significantly higher weight of plants and grain yield, shows that symbiotic N fixation was not enough for optimum bean yield.

5. Effect of N levels and Rhizobium on Phaseolus vulgaris

The effect of a comprehensive range of nitrogen (N) fertilizer levels on the symbiotic and yield responses of beans *Phaseolus vulgaris* L., cultivar Bonita) was studied in a field experiment planted in a Mollisol in southern Puerto Rico. A split plot design was used where the whole plots were the six N replications, and subplots were the inoculation treatments. The application of N significantly influenced the nodule number and nodule dry weight at 4 and 8 weeks after planting, respectively. Although bean yields tended to increase with greater N applications, no significant differences were observed. Inoculation with *Rhizobium* significantly increased nodule number and nodule dry weight at both sampling dates.

6. Nodulation survey of local legumes

The flora of Puerto Rico is well described, and includes more than 200 species of legumes. However, only limited information regarding the nodulation status of legumes growing in Puerto Rico is available. A survey of the nodulation status of legume plants was initiated in 1982 and continued until 1985. Table 4 shows the list of plants found lacking nodules, while table 5 indicates nodulated plants that had not been reported previously.

Table 4. Legume species recorded as non nodulating under Puerto Rico conditions

Species	Subfamily
Adenanthera pavonina	Mimosoideae
Caesalpinia bonduc	Caesalpinioideae
Caesalpinia coriaria	Caesalpinioideae
Caesalpinia decapetata	Caesalpinioideae
Cassia alata	Caesalpinioideae
Cassia hirsuta	Caesalpinioideae
Delonix regia	Caesalpinioideae
Stahilia monosperma	Caesalpinioideae

Table 5. New records of nodulated legumes in Puerto Rico

Species	Subfamily
Brya ebenus	Papilionoideae
Canavalia nitida	Papilionoideae
Cassia aeschynomene	Caesalpinioideae
Corynella paucifolia	Papilionoideae
Inga fastuosa	Mimosaoideae
Inga quaternata	Mimosoideae
Mimosa ceratonia	Mimosaideae
Ormosia krugii	Papilionoideae
Pictetia aculeata	Papilionoideae
Piptadenia flava	Mimosoideae
Pithecellobium arboreum	Mimosoideae
Rudolphia volubilis	Papillonoideae
Sabinea punicca	Papilionoideae
Vigna ambaensis	Papilionoideae

These results confirmed previous reports that a high percentage of Caesalpinioideae lack nodules. Although some of these species are fast growing trees, they should not be recommended for agroforestry purposes, since they compete with cultivated crops for available soil N. *Rhizobium* strains were isolated from the above mentioned nodulated legumes. They were tested for nodulation with siratro under aseptic tube conditions and added to the BNF Laboratory *Rhizobium* germplasm collection.

7. Sequential cropping in soybeans

Several possibilities are available for soybean production in tropical areas concerning the number of crops grown per year, because of the availability of cultivars from different maturity groups. In order to evaluate these possibilities, Dr. S. Cianzio started field experiments at Isabela and Lajas Research Centers to compare different planting dates and protein yields of cultivars from different maturity groups. This provided the opportunity to observe the effect of inoculation of different dates and cultivars under tropical conditions. Bimonthly experiments were planted with soil applied (granular) inoculant prepared strain USDA I-110SN of R. japonicum. Plants were sampled 4 to 6 weeks after planting, nodules counted and its contains plated on YEM with and without streptomycin to determine the percentage of nodules resulting from the inoculant strain. Results indicate that a large number of nodules were formed by the marked strain.

EDUCATION AND TRAINING ACTIVITIES

The lack of personnel trained in the technical aspects required for research and application of BNF technology constrains fuller utilization of inoculants in agricultural production. Therefore, education, training and dissemination of technical information was a major output of the BNF Project.

Formal instruction aspects can be divided into graduate and undergraduate levels.

a. Graduate Level

As member of the University Faculty, the P.I. of the Project was chairman and member of the graduate committee of three graduate students. During the course of the Project, three graduate students completed their requirements for a Master of Science degree, and a fourth student started her graduate program (see graduate students list, Appendix III).

During 1983, the University of Puerto Rico approved a new graduate course entitled "Biological Nitrogen Fixation" (CFIT 6645), on a temporary basis, and the course was taught by the P.I. during the second semester of 1983. The course was approved

as a permanent one as "Advances in Biological Nitrogen Fixation" during 1984, and taught as such during 1985. It will be taught in the future at every other year. Several graduate students have also completed Special Projects on Soil Microbiology.

b. Undergraduate level

Since 1981, the principal investigator has taught once a year the Soil Microbiology course (AGRO 4007). The course ws described inthe University catalog, but had not been taught for many years. The course stresses Biological Nitrogen Fixation and laboratory sessions cover applied aspects of BNF technology. To help further in the instruction of these aspects, a spanish preliminary edition of "Biological Nitrogen Fixation" written by the Project Leader was prepared and is available both at the main library and the Agricultural library. The possibility of including Soil Microbiology as a required course for the students of the Soils Program is under consideration of the Curriculum committee.

c. Other activities

With the cooperation of Dr. A.G. Wollum (NCSU) and Dr. W.C. Stearn (INTSOY), a short training workshop to identify Rhizobium strains using microaglutination techniques was held in October, 1980 at Mayaguez. Ms. Peggy Musselwhite (Department of Soils, (NCSU) was the instructor. As a result, the research assistants of both projects (INTSOY and BNF) and one graduate student were trained to perform serological identifications.

During the course of the BNF project, audiovisual equipment and materials were purchased, such as slide sets from the American Society of Agronomy, Int. Rice Res. Institute and FAO. Furthermore, slides prepared under the project are utilized for teaching, training and conferences. Further training and related activities are covered under section linkage and cooperative activities.

STATE OF THE ART (SOTA) DOCUMENTS

Under grant AID/csd-2857, the preparation of four SOTA manuscripts was responsibility of the University of Puerto Rico. The present grant was responsible for the editing and publication of those documents. Since the Bean SOTA was only at a draft stage and a BEAN CRSP publication was under way, the publication of this SOTA was cancelled. The Mungbean document reviewed for publication by Dr. J.M. Poehlman (University of Missouri, Columbia) was published in March, 1982. Authors Morton and Smith indicated their appreciation for the excellent editing work of Mr. Poehlman. The pigeonpea SOTA was edited by Dr. M. Lugo López and published in November, 1982, after several printing problems and delays at the printing shop (Antillian College Press). The SOTA's documents were distributed locally, to AID Missions and to interested scientists. During 1983, 1984 and 1985 there was a continuous demand for these publications. The greatest number of requests were from underdeveloped countries, and in particular for the pigeonpea publication, from India. Copies are still available and its distribution will continue upon request.

AZOLLA WORKSHOP

During the Biological Nitrogen Fixation Workshop at CIAT, Cali, Colombia, in March 1981, a group of scientists working on Azolla, agreed that there was a need to have a separate meeting dealing exclusively with Azolla in relation to Biological Nitrogen Fixation. The University of Puerto Rico, through its BNF Project and Title XII Strengthening Grant Program, offered its facilities and undertook the organization responsibilities through Dr. Eduardo C. Schroder, BNF Project Leader.

The workshop was organized with the collaboration of an International Program Panel, a steering committee and practical aspects handled by the organization committee. It was held from 17th to the 19th of November 1982. Several international organizations were asked to sponsor the meeting, but none was able to contribute. Therefore, organizational expenses were covered with monies from the Title XII and BNF Project (USAID), the Puerto Rico Rice Corporation (Secretary of Agriculture) and local private companies. Workshop attendance was approximately 50 persons representing 12 different countries. Over 20 research papers, ranging from basic Azolla studies to applied aspects were presented duringthe first two days of the workshop. A field trip to the rice growing area was held. Different aspects of land preparation, planting and harvesting were demonstrated, and a tour of the rice mill was conducted. In summary, the First International Workshop on Azolla was very successful, and provided an opportunity to share and exchange knowledge and professional interests.

During the workshop, it was agreed that Dr. W.S. Silver (Florida) and Dr. E.C. Schröder (UPR) would edit the Proceedings of the Workshop. An agreement was reached with Martinue Nijhoff/Dr. W. Publishers (Netherlands), and the book was published in November 1984. Copies of the Proceedings were distributed among the atendants and authors during 1985.

CONSULTANCY, LINKAGES AND COOPERATIVE ACTIVITIES

The Soil Microbiology Laboratory facilities at UPR-RUM were used by two USAID sponsored programs (BNF-UPR and INTSOY-University of Illinois) until August 1983, when Dr. Chris Stearn (INTSOY Microbiologist) was transferred to AVRDC (Taiwan) in order to be closer to the Asian region, where soybean is in stronger demand than in Latin America. The last aspects of laboratory work were carried on by Mrs. Magali Rodríguez, and INTSOY finished its activities in Puerto Rico in December, 1984. This represented an important reduction in BNF capabilities for Puerto Rico and Tropical America.

Dominican Republic

Due to its close proximity, the existence of ongoing projects and established communication channels, a strong linkage has been established between the UPR-BNF Project and the Dominican Republic.

In response to a letter by Dr.César E. López (Secretaría de Agricultura, Departamento de Investigaciones Agropecuarias) requesting the review of Research Projects on Biological Nitrogen Fixation, Dr. Eduardo C. Schröder visited the Dominican Republic between July 18 and 24, 1982. Ongoing and future research projects involving leguminous trees,forage and grain legumes were discussed with scientific personnel from the Secretaría de Agricultura and three of the four Universities with Agronomy Programs. Since the lack of personnel is a major limiting factor, scholarships to work on BNF towards a M.S. degree at UPR-RUM were offered. As a result, several applications were received at the Graduate Studies Office of UPR, and Mr. Yovanny A. Velázquez, an agronomist working for the Secretaría de Agricultura, was accepted. He started his M.S. degree program in 1983 and completed his graduate work by December, 1984.

During 1983, the Research Director and the Director of the San Cristóbal Research Center requested further assistance, and a training course and workshop was organized. The event was coordinated by Lic. Elfrida Pimentel, of the Secretaría de Agricultura. The activity was held at the facilities of INDOTEC (Instituto Dominicano de Tecnología Industrial), where excellent laboratory and classroom facilities were available. The First Caribbean Rhizobium meeting was a cooperative activity among the FAO Regional Office in Santiago, Chile, the UPR Bean/Cowpea CRSP, NifTAL Project, and the University of Wisconsin (Madison). The workshop was held from August 6 to 10, and the international invited speakers included: Rosemary S.

Bradley (Colombia), David M. Hubbell (University of Florida), Robert A. Kluson (UPR, Mayaguez), Juan C. Rosas (University of Wisconsin), Joann P. Roskoskl; (NiFTAL), and Eduardo C. Schröder (UPR, Mayaguez). Some of the invited Caribbean scientists were not able to attend, but Haiti and Barbados were represented. Local participants were over 30 in number, representing several research groups and institutions. Therefore, the Caribbean Basin was represented by Barbados, Haiti, Dominican Republic and Puerto Rico.

In order to establish more permanent links among the Caribbean countries with established Rhizobium research groups, a meeting was held with the international delegations. The consensus was that a group should be organized and that it should be part of the Latin American Rhizobium Association (ALAR). Dr. Eduardo C. Schröder was named coordinator. The petition was taken to the Brasil ALAR meeting, where it was approved. In order to maintain a linkage, the BNF Project sponsored the publication of a regional circular sheet with pertinent information; this is published both in spanish and english. Further steps are needed to form a regional network for research, training and information sharing. The present UPR-BNF Laboratory could serve as the regional center for this network. These activities depend on obtaining the necessary funds from USAID and other international agencies.

In 1985, an agreement was signed by the Secretaría de Agricultura, the Universidad Autónoma and the UPR to conduct research in BNF under a USDA/AID grant.

Guatemala

In 1981, the Instituto Centro Americano de Investigación y Tecnología Industrial (ICAITI) requested consulting services in the area of BNF through AID-ROCAP. As a result, Dr. Eduardo C. Schröder visited Guatemala during January 18th to 23rd, 1982.

The Division of Applied Research of ICAITI, headed by Dr. Carlos Rolz has been working for several years in the field of Biotechnology. They are interested in pursuing the field of BNF and help Central America by developing technology in this field. The visit included inspection of facilities, presentation of seminars on BNF and inoculant production, discussion of projects and visits to ROCAP, ICTA (Instituto de Ciencia y Tecnología Agrícola) and University of San Carlos at Guatemala. The establishment of a network for research in BNF in Central America was discussed, but in order to develop BNF capabilities at ICAITI, technical assistance in the form of training work with Rhizobium and consultants in inoculant production technology are needed. More recently,

a reseach project on R. phaseoli inoculant production was approved by the NAS, and Roberto de León is incharge of the Project.

Costa Rica

During May 1983, Dr. Carlos Ramírez (University of Costa Rica),organized a four week training course in Rhizobium technology. The event was sponsored by UNESCO, UNEP, ICRO, CONICIT, and NiFTAL. The course was attended by 17 students from the Caribbean, Central and South America,and eight countries were represented. Dr. Eduardo C. Schröder was among the invited lecturers. The course provided anunique opportunity to interact with young professionals interested in Rhizobium. Aspects of our BNF Project and work in Puerto Rico were explained. The trip helped to establish a link with the University of Costa Rica and CATIE for future activities.

Haiti

The National Academy of Sciences sponsored a trip by Dr. Eduardo C. Schröder to assist Dr. Jean Fenel Felix (Agronomy School of Haiti) to rewrite a research proposal to study pigeonpea inoculation response. The NAS approved the grant, and as a result, a project review was done during April 21- 25, 1985. Furthermore, Dr. F. Felix and his research assistant received training in Rhizobium serological identification techniques at the BNF Laboratory (UPR) during November, 1985.

ALAR and other linkages

The ALAR organizes a meeting of Latinamerican Rhizobium workers every two years. During the BNF Project, the P.I. was able to attend 2 of these important regional meetings. The first one was the XI RELAR (Reunion Latinoamericana de Rhizobium),held at Lima, Peru in October, 1982, and the second one was the XII RELAR held at Campiñas, Brasil. These meetings were a good opportunity to initiate and strengthen linkages between UPR and Latin America, and several requests for information, strains and seeds were distributed.

OTHER ACTIVITIES AND ACHIEVEMENTS

The principal investigator, as a staff member of the University of Puerto Rico, participated in Departmental and Faculty meetings. Since 1982, he has been member of the Curriculum Committee. In addition to being Chairman of graduate students in the Department of Agronomy, the P.I. has been member of other graduate students committes and serve as graduate studies representative in final exams for M.S. degrees. Other general activities include talks and informal reports on BNF activities to short training interns and visitors.

In order to establish a permanent research group in BNF at the University of Puerto Rico, several proposals and preproposals were prepared yearly. Among those, the following projects were funded:

Title	Funding Institution	Year
1. Effect of soil pH and host genotype on the nodulation and nitrogen fixation of *Leucaena* spp.	USDA/TAD	1983
2. Increasing *Phaseolus vulgaris* yields in the Dominican Republic by optimizing the host/strain (R. phaseoli symbiosis)	USDA/AID	1985

In 1983, the building where the BNF Laboratory is located was remodelled according to our design, and the new facilities have helped to organize separate laboratories, instruments room, offices and sterilization room. Some new instruments (gas chromatograph, spectrophotometer, pH meter, ultrasonic cleaner, etc.) were added to the laboratory with funds provided by the University of Puerto Rico.

GRANT IMPACT AND FUTURE PLANS

The grant was successful in establishing and developing the University of Puerto Rico institutional response capacity in the area of Biological Nitrogen Fixation by Tropical Legumes. As a result, our knowledge on Rhizobium inoculation of legumes was expanded, the educational capability of the Department of Agronomy and Soils was improved, and the advisory ability of the University of Puerto Rico was increased.

The University of Puerto Rico, through its College of Agricultural Sciences, is interested in maintaining and expanding the activities related to BNF. The University supports part of the Program by paying the P.I. salary, and full support is given to search and obtain additional funds from other institutions or possible cooperative linkages. Besides research, further training at the M.S. level and training courses are encouraged. However, long term financial backing is needed for such activities as legume seed germplasm program; Rhizobium strain collection, education and training of foreign students and the development of the BNF Laboratory as a BNF resource center for the Caribbean.

APPENDIX I. MEETINGS AND TRIPS

During the BNF Project, the P.I. participated and attended to the following meetings:

1980

1. P.I. SEA/CR AID Nitrogen Program. April 10–11, Kansas City.

2. ASM 1980 Annual Meeting. May 11–16, Miami Beach, Florida.

3. AID and BNF Consortium Representatives Meeting. June24–27, Washington, D.C. and Ithaca, N.Y. Dr. Luis M. Cruz Pérez, Director of Agronomy Department, also attended.

4. BNF Consortium Representatives Meeting. November 10 –11, Raleigh, North Carolina.

5. First Conference on Fertility Transfer. December 9– 11, Mayaguez, Puerto Rico.

1981

1. Biological Nitrogen Fixation Technology for Tropical Agriculture Workshop and Nitrogen Cycling in Ecosystems of Latin America and the Caribbean. March 9–13 and March 16–21, Cali, Colombia.

2. International Training Course in Rhizobium Technology (Niftal). June 13–28, Chapingo, Mexico.

1982

1. BNF Consortium Representatives Meeting. Technological Assessment of BNF Panel Sessions. Visit to NifTAL. January 10– 15. East–West Center and Niftal Project, University of Hawaii, Hawaii.

2. Guatemala. BNF Consulting Services. January 18– 23. ICAITI–ROCAP. Guatemala City, Guatemala.

3. BNF Limiting Factors Workshop. February 22 23. Washington, D.C.

4. Dominican Republic. Technical assistance request. July 18—25. Santo Domingo, Dominican Republic.

5. XI RELAR (Reunión Latinoamericana de Rhizobium). October 24-29, Lima, Perú.

6. SOPCA — (Sociedad Puertorriqueña de Ciencias Agrícolas). November 5. Aguadilla, Puerto Rico.

7. Practical Applications of Azolla for Rice Production (Workshop). November 17-19. Mayaguez, Puerto Rico.

1983

1. Southern Regional S -170 Committee Meeting. January 16-18, Atlanta, Georgia.

2. Regional Training Course on "Symbiosis between Rhizobium and Tropical Legumes of Agricultural Importance". May 15—20, San José, Costa Rica.

3. 9th North American Rhizobium Conference. June 17-23, Ithaca, New York.

4. Haiti. Assist Dr. J.E. Felix, NAS Grant Proposal. June 30 -July 2. Port au Prince, Haiti.

5. 5th International Symposium on Nitrogen Fixation. August 28-September 3, Noordwijkerhout, The Netherlands.

6. Caribbean Food Crops Society. 19th Annual Meeting. September 12-15, Mayaguez, Puerto Rico.

7. SOPCA. (Puerto Rican Society of Agricultural Sciences) Annual Technical Meeting. October 28, Mayaguez, Puerto Rico.

1984

1. First Caribbean BNF Workshop. August 6 to 10, Santo Domingo, Dominican Republic.

2. SOPCA (Puerto Rican Society of Agricultural Sciences). Annual Technical Meeting. October 11, Mayaguez, Puerto Rico.

3. BNF Biotechnology Meeting (Latinamérica). First Workshop (UNDP, UNESCO, UNIDO), October 18, 19, 20, Campinas, Brasil.

4. XII RELAR (Reunión Latinoamericana de Rhizobium). October 21–26, Campinas, Brasil.

1985

1. Agreement Implementation. February 22–27, Santo Domingo, Dominican Republic.

2. Advisory visit. April 21 25 (NAS BOSTID). Haiti.

3. 6th International Symposium on Nitrogen Fixation. August 4–10, Corvallis, Oregon.

4. 10th NAPC and BNF Limiting Factors Workshop. August 11–21, Maui, Hawaii.

APENDIX II. BNF PUBLICATIONS (1980-1986)

Schröder, E.C., 1981. Efecto de la inoculación de "zarcilla" Leucaena leucocephala, Lam, de Wit) en un oxisol en Puerto Rico. Ann. Proc. SOPCA 1981 p. 9 (Abstract).

Morton, J.F., Smith, R.E., and Poehlman,J.M., 1982. The Mungbean. Special Publication, Department of Agronomy and Soils, University of Puerto Rico, Mayaguez, P.R., 135 pp.

Morton, J.F., Smith, R.E., Lugo-López, M.A., and Abrams, R. 1982. Pigeonpeas: (Cajanus cajan Millsp.): A valuable Crop of the Tropics. Special Publication, Department of Agronomy and Soils, University of Puerto Rico, Mayaguez, P.R. 123 pp.

Schröder, E.C., and Gaztambide, M.A., 1982. Results of an "INLIT" trial with Leucaena leucocephala Lam. de Wit. Proc. XI Latinamerican Rhizobium Meeting (RELAR). Lima, Perú, October 1982 p. 145-158 (S,e).

Schröder, E.C., 1983. La Fijación Biológica del Nitrógeno y el Proyecto BNF del Departamento de Agronomía y Suelos (RUM). Primera parte. Revista del Colegio de Agrónomos de Puerto Rico, p. 7 (S).

Schröder, E.C., 1984. La Fijación Biológica del Nitrógeno y el Proyecto BNF del Departamento de Agronomía y Suelos (RUM). Segunda Parte. Revista del Colegio de Agrónomos de Puerto Rico, p. 7 (S).

Schröder, E.C. 1984. Delonix regia Confirmation of its non-nodulating character. In: Advances in Nitrogen Fixation Research. Veeger, C. and Newton, W. E. (eds) M. Nijhoff/Dr. W. Junk, (PUDOC), The Hague. p. 359.

Silver, W.S. and Schröder, E.C. (eds). 1984. Practical Applications of Azolla for Rice Production. Martinus Nijhoff/Dr. W. Junk Publ., The Hague, Netherlands.

Gaztambide, M.A. de, and Schröder, E.C. 1984. Effect of toxic substances of leucaena seed on Rhizobium. Ann. Proc. SOPCA 1984 p.4 (Abstract) (S).

Velázquez, Y.A., and Schröder, E.C. 1984. Response of Phaseolus vulgaris to inoculation with Rhizobium phaseoli. Ann. Proc. SOPCA 1984 p.4 (Abstracts) (S).

Schröder, E.C. 1984. The role of microcomputers in rhizobiology laboratories. Proceedings of the XII RELAR (Reunión Latinoamericana sobre Rhizobium). Campinas, Brasil. (S,e).

Schröder, E.C. and Gastambide, M.A. de, 1984. Evaluation of inoculation methods for Cajanus cajan in Puerto Rico. Proceedings of the XII RELAR (Reunión Latinoamericana sobre Rhizobium). Campinas, Brasil. (S,e).

Schröder, E.C., 1985. Rediscovery of nodulation in Ormosia and Neorudolphia two native legumes of Puerto Rico. J. Agric. Univ. P.R. 69 (2): 229.

Schröder, E.C., 1986 Recent Advances in Leucaena Research. 4th Annual Meeting of the OAS/Caribbean Leucaena Project. February, 1986. St. Lucia. 17 pp.

Vicente, N.E., Acosta, N., and Schröder, E.C. 1986. Reaction of Leucaena leucocephala to population of Meloidogyne incognita and M. javanica from Puerto Rico. J. Agric. Univ. Puerto Rico 70: (in press).

Mangual Crespo, G., Kluson, R. and Schröder, E.C. Effect of various nitrogen levels and Rhizobium inoculation on yields of native white bean (Phaseolus vulgaris L.). J. Agric. Univ. P.R. 70: (in press).

THESIS

Student name	Year Presented	Thesis Title
Ismael Matos Torres	1983	Evaluation of *Rhizobium* sp. strains for their nitrogen fixation capacity with pigeonpeas (*Cajanus cajan* Millsp) variety 2B-Bushy.
Yovanny A. Velázquez	1984	Inoculation responses of beans (*Phaseolus vulgaris* L.) variety Arroyo Loro No. 1 to inoculation with *Rhizobium phaseoli* in Lajas, Puerto Rico.
Raquel Robledo Rosario	1985	Effect of different mulches on soil temperature and moisture, and on the *Rhizobium phaseoli*-*Phaseolus vulgaris* L. symbiosis.

APPENDIX III

PROJECT PERSONNEL

Staff	Position	Months served
Myrna Alameda	Research Assistant	10/80 – 12/85
Rodulio Caudales	Instructor	7/80 – 7/82
Luis Cruz Pérez	Project Leader/ acting	1/79 – 3/80
Robert Kluson	Research Assistant	12/83 – 6/85
Aida Méndez	Research Assistant	1/79 – 7/79
Susan Padilla	Secretary	1/79 – 9/80
María A. Pagán	Secretary	1/81 – 12/85
Doris B. de Rivera	Research Assistant	5/80 – 9/80
Miguel Rivera	Laboratory Aid	1/79 – 7/85
Eduardo C. Schröder	Project Leader/ Microbiologist	3/80 – 12/85

GRADUATE STUDENTS

Name	Country	Program status
Ismael Matos	P.R., USA	M.S. degree, 1983
Jacqueline Lazu	P.R., USA	Thesis research
Sarah Lugo	P.R., USA	Withdrawn
Raquel Robledo	P.R., USA	M.S. degree, 1985
Yovanny A. Velázquez	Dominican Republic	M.S. degree, 1984

APPENDIX IV

VISITORS LIST

Name	Institution/Country
J.M. Poehlman	University of Missouri
A.G. Wollum	North Carolina State University
D.J. Sammons	University of Maryland
L. Frederick	DBS/AGR, AID, Washington, D.C.
P. Musselwhite	North Carolina State University
B.L. Smith	Ohio State University
F.T. Davis, Jr.	Texas A & M University
G.W. Akin	Tennessee Valley Authority
N.W. Axinn	Michigan State University
J.L. Brewbaker	University of Hawaii
D.G. Farías	ICRISAT, India
R.H. Gibson	Tenneseee Valley Authority
R.H. Howell	University of Illinois
J. Jackobs	University of Illinois
H. Kauffman	University of Illinois/INTSOY
H H. Mundel	Agriculture Canada, Canada
Agronomy Dept. Review Team	(Several members and institutions)
INTSOY Review team	(Several members and institutions)
F. Bliss	University of Wisconsin
A. Liogier	Botanical Garden, UPR
R.A. Jones	University of Georgia
V. Chew	USDA, Gainesville, Florida
C.G. Manos	Cornell University
G.S. Rahi	Florida
Rep. D. Bereuter (Nebraska)	U.S. Congress, Foreign Affairs Committee
M.W. Michaud	College of the Virgin Islands, St. Croix

M. Rosario	SEA, Dominican Republic
M. Olivero de Sánchez	SEA, Dominican Republic
R.J. Baker	Ministry of Agriculture, Jamaica
J. Fenel Félix	Faculty of Agronomy, Haití
P. Mathieu	Port au Prince, Haití
J. Spinger	Bahamas Agricultural Research Center
R. Williams	Ministry of Agriculture, Barbados
J. Peisajovich	Universidad Veracruzana, México
L. Tergas	CIAT, Colombia
J. Jones	USDA-SEA, Florida
N. Cavallaro	Chapingo, México
S. Zuloaga	EAP, Honduras

APPENDIX V

EXPENDITURE REPORT

Function/Activity	Amount ($)
Salaries and wages	$ 192,132.35
Fringe Benefits	32,805.89
Travel	31,939.40
Equipment	11,943.07
Books and Journals	9,225.57
Materials and Supplies	81,395.18
Unliquidated obligations	558.54
TOTAL EXPENDITURES	$ 360,000.00
AMOUNT AUTHORIZED	$ 360,000.00
BALANCE	$ 0.00

* As February 28, 1986