Proj 9311004 (2)
PN - PD-AAD-240-B1

RESEARCH PROJECT STATEMENT

N March 1976

24p

1. PROJECT SUMMARY

a. Statistical

Project Title:	Nitrogen Fixation by Associative Grass-Bacteria Systems
New or Extension:	New
Contractor and Address	Center for Tropical Agriculture, University of Florida Gainesville, Florida 32611
Principal Investigators:	Dr. Rex L. Smith & Dr. S. C. Schank
Duration:	Four Years
Request RAC Authorization:	Three Years
Total Estimated Cost (3yr):	$890,000 (three years) FY 77 -- $270,000 FY 78 -- 300,000 FY 79 -- 320,000
Project Manager:	Dr. Lloyd Frederick

b. Narrative:

The major objective of this project is to develop a dependable grass-bacteria system that will fix significant amounts of N from the air for the crop. Greater knowledge and understanding of the grass-bacteria system and how it functions must be obtained. The grass-bacteria associations appear to be much more sensitive to genetic and environmental factors than the legume-rhizobia system. As a result, they have not been controlled and utilized by man. Development of dependable grass-bacteria N-fixing systems will permit their management and the exploitation of their enormous potential for increasing food production.

The University of Florida has organized a team of scientists to investigate the potential of associative biological nitrogen fixation to reduce significantly the need for fertilizer nitrogen in grass crops. Examination of a wide range of grass genotypes has detected N-fixation capability in many cases. Nitrogen fixing bacteria, especially Spirillum lipoferum,

have been found on and within the roots. Measurements on some root segments indicated significant N fixation but the rate varied with plant genotype and the environmental conditions. Tropical temperatures (about 29°c) were especially favorable. The most effective combinations of plant genotype and bacterial strain remain to be determined. In greenhouse and field experiments, sometimes significant yield increases were found, but the results have been inconsistent in different trials. A dependable, reproducible system has yet to be found.

To attack the problem this research project is proposed to (1) search for more dependable and effective associations; (2) identify the plant and bacterial characteristics necessary for effective association; (3) define critical environmental conditions for effective nitrogen fixation for each association; and (4) measure the amount of nitrogen fixed and the value of the fixed nitrogen in replacing fertilizer nitrogen. When a dependable grass-bacteria association is developed, it should be tested in LDC farming systems.

In TAB's recently developed strategy paper on "Biological Nitrogen Fixation," the grass-rhizosphere associative nitrogen fixation system was recognized as a high priority research area with tremendous potential and high risk with regard to payoff. This project represents the major thrust proposed for AID support in this area. Supplementary programs supported by AID are the NAS program with Brasil for exchange and support of scientific personnel and a smaller research effort on wheat at the University of Nebraska. These projects around out AID's program in associative nitrogen fixation at this time, but positive results may indicate need for additional efforts, especially in transferring technology to LDC's.

2. RESEARCH PURPOSE AND EXPECTED PRODUCTS:

a. Project Purpose:

The major objective of this research is to critically study tropical and sub-tropical grasses for the purpose of developing dependable associative grass-bacteria nitrogen fixing systems so grass crops can be produced with less fertilizer. When dependable systems are developed, determine if these systems can be managed and used in farming on a practical basis.

Expected outputs:

1. An associative grass-bacteria system that consistently fixes significant amounts of nitrogen. Nitrogen-fixing activity will be measured in large numbers of grass genotypes to find grass root systems giving in-

creases in yield and nitrogen uptake. Genotypes and associations will be sought that show consistent and dependable N-fixation in significant quantities.

2. Grass genotypes and bacterial strains with characteristics necessary for effective N-fixing association, and more effective combinations of grasses with bacteria. Isolates of bacteria that form dependable, effective associations with grass will be sought.

3. Proper environmental conditions, such as temperature, O_2 level, light, other nutrients and inoculation of the proper bacteria, for a more effective N-fixing association. When the conditions needed for a dependable effective association are established, selections may be made to broaden tolerance to environment.

4. Nitrogen fixed biologically that is equivalent to 30 to 100 Kg fertilizer N/ha or more. Larger amounts of N-fixation will be sought, but enhancement of nitrogen fixation in grasses in these amounts would have tremendous economic and food production potential for farmers, especially those on family farms.

5. Incorporate a dependable grass associative N-fixation system into LDC farming systems. This output can not be implemented until research has developed a dependable system. If the research proposed here is successful in domesticating the grass-bacteria N-fixation system, it would take at least 2 to 3 years to establish its dependability and value, therefore this objective would primarily be in the third year, or later. When a dependable system is developed, additional work may be required to extend the system to other species or cultivars.

3. SIGNIFICANCE AND RATIONALE FOR THE RESEARCH

"Studies and research on the practical aspects and possibilities for domestication of grass-bacteria rhizosphere associations" was identified as a high priority project area in the TAB strategy report on Biological Nitrogen Fixation. This project is directly supportive to key project areas for AID assistance in improvement of basic food crops and the management of soils to provide adequate nutrients for crops.

Grass crops dominate world agriculture. Total production of cereals is about 10 times that of grain legumes, and the cereals contain about 4 times as much total digestible protein. If associative nitrogen fixation can

increase the yield and N uptake of grass crops significantly, the food potential is tremendous. Dr. R. Desrosiers put it this way, "it would be a serious error to fail to support research on nitrogen fixation in the Graminaceous plants. I feel that the discovery of the symbiotic association between nitrogen-fixing bacteria and the roots of grass plants is an event of far greater importance than the discovery of high-lysine maize." "The fact that nitrogen fixation has been demonstrated on forage grasses, maize, wheat, sorghum and millet greatly improves the prospects for successful incorporation of this capability into varieties of crop plants." "By assisting in this research AID would be making a most important contribution towards the solution of the energy problem and the increase of food production, as well as a contribution to minimum-input agriculture."

The recent discovery of this grass-bacteria rhizosphere associative nitrogen fixation system by Dr. Dobereiner in Brasil has been confirmed by many other workers. Grasses, in association with bacteria, can use atmospheric nitrogen for some of their nitrogen requirement, but the systems have been erratic and the actual amount of nitrogen made available has not been determined.

For these grass systems to be useful, they must be dependable and reproducible by proper management. Susceptibility of grasses to establishment of this association appears to be variety specific, but difficulties have been encountered in getting the same results in different trials. In preliminary tests in Florida, small plots of guinea grass and pearl millet inoculated with Spirillum gave significant yield increases. Some other grasses did not respond to inoculation in these trails. Research to determine the dependability and repeatability of grass-bacteria associations that fix nitrogen effectively in the field is urgently needed.

This project is part of the AID strategy in biological nitrogen fixation. It represents the major research effort in development of the associative grass-bacteria system, but is augmented by the exchange and support of scientific personnel with Brasil through the National Academy of Sciences and the Brasilian program on "Basic Research in Nitrogen Fixation." This new project will be linked with a smaller effort in wheat under the research contract at University of Nebraska. Studies on the associative grass system complement studies on biological nitrogen fixation supported by AID on the symbiotic legume-rhizobia systems through the following projects:

(a) more effective rhizobia for tropical legumes (University of Hawaii); (b) better soybean rhizobia (INTSOY); (c) Rhizobium germplasm collection, preservation and evaluation (ARS, USDA, Beltsville) and (d) overcoming factors limiting the rhizobia-legume system (Grants through CSRS). State-of-the-art and other studies are to be carried out through the Consortium on Soils of the Tropics. Symposia, workshops and training activities are supplementing the research efforts on biological nitrogen fixation.

4. PLANS FOR RESEARCH LINKAGES

The linkages between various TAB activities on biological nitrogen fixation are described in the discussion of strategy in the previous section. Drs. Schank and West of the University of Florida are members of the Advisory Committee for the Program for International Cooperation in Training and Basic Research on Nitrogen Fixation in the Tropics. This program is located in Brasil at Rural University, Km 47, Rio de Janeiro under the direction of Dr. Johanna Dobereiner. AID has a Task Order to support the exchange of scientific personnel and supplies in this program through NAS. Linkages with the research activities of Dr. Burris at University of Wisconsin and Dr. Evans at Oregon State University have been established, as well as with USDA scientists at BARC and Puerto Rico, and with the Southern Region Work Group on nitrogen fixation in grasses.

Personal contacts have been made with scientists at CIAT, IITA, IRRI and ICRISAT, and with UNDP, FAO personnel. Collections of grass roots from which N-fixing bacteria were isolated have been made in Brasil, Ecuador, Venezuela and several west African countries. Other linkages are planned with LDC's and international agencies as opportunities develop. A workshop is planned at University of Florida in April, 1976 to bring workers together, discuss progress, methods and research linkages.

5. PLAN TO FACILITATE UTILIZATION OF RESEARCH RESULTS

This project is designed to explore the potential of the associative grass-bacteria nitrogen fixation system. The major objective is to develop a reliable system that contributes significant amounts of nitrogen to the grass crop. Until this system(s) is developed, utilization of results by the farmer is not feasible. When a promising system is developed, its utility will be evaluated at various locations.

The training provided to graduate students and scientists working in this scientific frontier is extremely important. The University of Florida staff

have developed competence recently through their work on the grass-bacteria rhizosphere supported by funds from their own institution, USDA and an AID small research project. The graduate students, post-doctorals and technicians will receive training; some of these will come from LDC's. When a dependable grass N-fixing system is established, other training opportunities can be considered.

Because cereal crops are grasses widely grown in agriculture everywhere, if a workable system can be attained, no difficulty in utilization is anticipated. Information will be provided to regional bureaus and the International Centers. Publications and symposia also will be used to disseminate information.

6. MANAGEMENT CONSIDERATIONS

A. The co-Directors of the Project responsible for technical and managerial oversight are Dr. Rex Smith and Dr. Stanley Schank, Department of Agronomy, IFAS, University of Florida, Gainesville. The Project Officer from USAID/W is Dr. Lloyd Frederick (TA/AGR) who will monitor progress, scientific quality and assure that the research efforts are carried out in accordance with the goals of AID.

B. The research contractor (CTA/University of Florida) was selected after informal discussions with several scientists from other universities, NAS and AID staff. This contractor was selected because no one else had: a) an organized research team of agronomists, crop breeders andphysiologists, soil microbiologist, and microbiologists; b) a cooperative and understanding attitude toward the needs and desires of AID and tropical areas; c) experience in field, greenhouse and laboratory studies already initiated in associative biological nitrogen fixation; d) a research effort with which AID could cooperate and utilize the capability already developed and e) the continuity of effort necessary to carry this project to the desired conclusion.

Financial contributions not funded by AID comprise a sizeable input into this project. USDA has a plant physiologist and two technicians who spend a majority of their time on this project. The University of Florida has three senior staff members (agronomist, crop breeder and micro-biologist) who spend a majority of their time on this project and four others (cytogeneticist, soil microbiologist, microbiologist and biochemist) who spend appreciable time on the project. These staff form the core of expertise and supervision for the junior staff supported by the project. Sub-contracts

are to be determined and managed by the contractor following approval by project officer and contracts officer, AID/W. Sub-contracts are planned to extend capability and to gain information on other soil conditions and plant environments. Regular on-site evaluations using non-project staff from regional bureaus, USDA, or universities are planned.

7. TECHNICAL REVIEW

Dobereiner and co-workers in Brazil have pioneered research on nitrogen fixation by grass-bacteria rhizosphere associations. Of particular significance was the discovery of the rhizosphere association of Azotobacter paspali with Paspalum notatum (bahiagrass) where they calculated N_2-fixation rates as high as 90 kg N/ha/yr. Another bacterial association, Beijerinkia indica with sugarcane also was reported. In these two systems, nitrogen fixation using washed roots was greater than that of the rhizosphere soil, suggesting that an intimate relationship existed between roots and bacteria, The bahiagrass association was reported to be grass-genotype specific. Acetylene reduction was later studied in several other species of tropical grasses comparing washed roots with rhizosphere soil. Acetylene reduction rates of over 700 nmoles/ (g root dry wt. X hr.) were measured for Bracniaria mutica and Pennisetum purpureum, about 300 for Digitaria decumbens and Panicum maximum, about 150 for Cynodon dactylon, but under 100 for the other forage species tested. Soil rates were insignificant.

Recent evidence indicates a N_2-fixing association which may assume great importance. Dobereiner and Day isolated Spirillum lipoferum from "Transvala" digitgrass (variety released recently from the Florida Experiment Station). Nitrogen increases were recorded in field-grown tropical grass in Brazil, as well as a potential nitrogen fixation of about 0.5 kg N/ha/day by Schank et al. Root cortical cells were observed to contain bacteria which resembled S. lipoferum in size and morphology. This cytological work suggested an associative symbiotic relationship between S. lipoferum and Digitaria decumbens

Corn (Zea mays) was reported by von Bulow and Dobereiner to associate with Spirillum and reduce acetylene at very high rates, up to 7124 nmoles/ (g dry roots X hour). Using the standard 3:1 conversion rate (C_2H_2/N_2 reduced), potential nitrogen fixation was projected to a maximum rate of 2 kg N/ha/day.

In Florida, Spirillum strains from Brasil have been used to inoculate grasses in the field for two years. In 1974, new plantings of inoculated "Transvala"

and Panicum maximum yielded about 150% of the uninoculated control. In 1975, inoculated pearl millet yielded about 120% of the uninoculated control. In this experiment, nitrogen fertilizer rates of 0, 20, 40, and 80 kg N/ha applied as ammonium nitrate were used in 8 replications only the 40 and 80 kg/ha fertilizer rates gave higher yield due to inoculation. In this study, about 40 kg of additional fertilizer N would have been required in the uninoculated plots to produce yields equivalent to those that were inoculated. Productivity data are especially important. These indicate that some fixed nitrogen can be used by the plant to improve productivity and also give good evidence for nitrogen fixation to support acetylene reduction measurements.

The use of the acetylene reduction technique to assay nitrogenase activity is well documented. The Dobereiner technique for treatment of root samples for use in the study of nitrogen fixation in grasses has been severely criticized because of extended preincubation at reduced oxygen levels (generally, 2% oxygen and lower for 12 hr). Measurements made with this low oxygen content of the gas mixture during preincubation probably promotes the accumulation of organic acids and other fermentation products. Burris at Wisconsin has found increases of a thousand-fold for the nitrogen-fixing organisms. Extrapolated N_2-fixation values from such data are therefore questionable and at best demonstrate potential N_2 fixation. Acetylene reduction has been measured by incubating roots in acetylene for a short time. Results have been highly variable even with replicates and when sampled at different times. More data are needed to show increased productivity and/or replacement of fertilizer nitrogen by fixed nitrogen in these systems.

Balandreau and Dommergues from France reported rates of 3000 nmoles/ (g dry root X hr) on Zea mays but only 40 nmoles/ (g dry root wt. X hr.) on Paspalum notatum.

Flooded rice plots at IRRI were reported to fix up to 63 kg N/ha. Most of this was attributed to non-symbiotic fixation in the soil. Water in planted paddies fixed only about 3 kg N/ha. Balandreau, et al reported that nitrogen fixation in rice was of the same magnitude as peanuts and that grassland appeared to much less active.

At Florida in 1975 grass roots were screened for nitrogenase activity. Highest acetylene reduction rates have been obtained with sorghum. High rates also were obtained with corn and pearl millet. Perennial tropical forage grasses have had lower activity rates than these grain producing grasses. Higher acetylene reduction rates were observed in Chloris guyana,

Panicum maximum and *Cenchrus ciliarus* than in "Transvala" digitgrass and *Hemarthria altissima*.

Erratic acetylene reduction rates appear to indicate that nitrogen fixation is very sensitive to environmental, soil and plant conditions in these systems. N_2-fixation ceased in plants when the seeds begin to fill; good fixation was obtained only when the plants were capable of rapid growth.

Cytological examination of buffelgrass roots revealed that both *Spirillum* and other rod-shaped bacteria were present in root cortical cells. Using fluorescent antibody techniques, the *Spirillum* were identified because *Spirillum lipoferum* antibodies reacted with *Spirillum* but not the smaller rod-shaped bacteria. This demonstrates that *Spirillum* and other bacteria can penetrate the root and leads to the hypothesis that an associative symbiosis exists. An intimate close relationship is necessary for the plant to supply energy efficiently to the bacteria for N_2-fixation.

To date, there are no reports of consistent repeatable data for nitrogen fixation by reconstituted associations of grass-bacteria in greenhouses or growth chamber studies.

8. RESEARCH PROJECT DESIGN AND METHODS:

A. Summary

Field inoculation results from Florida in 1974 and 1975 demonstrated that significantly higher yields could be obtained for *Spirillum* inoculation with some tropical and sub-tropical grasses (Guineagrass, digitgrass, and pearl millet). Some variable and unpredictable results in these replicated tests indicated that other important factors affecting root-bacterial associations and N_2-fixation must be defined. In order to evaluate the environmental and plant factors, experiments will be conducted initially under controlled conditions in greenhouses and growth chambers.

The first research objective is to find and develop effective and repeatable N-fixing associations. Many plant genotypes will be used to search for effective associations. Successful plant genotype specificity will be incorporated and tested using various *Spirillum* strains to develop more effective and dependable associations;

The second objective is to identify the plant and bacterial characteristics necessary for effective associations. Suceptibility of the plant to infection, mechanisms of infection, and nature of the association of bacteria and plant will be investigated along with nature of energy exchange and nitrogen transfer. Electron microscopy, fluorescent antibody and ^{15}N will

Third, adaptation of N_2-fixing bacteria to soil and environmental conditions needs clarification. Successful inoculation depends upon establishment of the bacteria in the soil. Ecological studies of specific bacteria populations will be conducted. Widespread natural distribution of N_2-fixing bacteria should permit selection for various soil types and plant species. Collection, classification and screening of bacteria from a broad range of conditions should provide organisms with a wide base of adaptability to soils and plants.

Fourth, measurements on the amount of nitrogen fixed and the value of the fixed nitrogen to the crop plant will be accomplished. Methods to accurately and realistically quantify N_2-fixation will be used to supplement acetylene reduction data. These will include inoculation trials, yield trials, N balance studies and in situ acetylene reduction assays.

Finally, when a dependable association is developed it will be tested in LDC farming systems. Enhancement of N_2-fixation may be achieved through inoculation, better genotypes, fertility control and/or crop rotation. Once this methodology is developed, it would allow rapid progress and application to LDC's.

B. Research Design and Methods - Detailed

1. Search for more dependable and effective plant-bacterial associations and to identify more effective N-fixing bacteria.

Grass-bacteria systems have been difficult to evaluate because of their undependable and erratic fluctuation during the season. To find and develop dependable effective associations in the laboratory or in the field is therefore of prime importance. Most of the other outputs in this project are contingent on having a system that works under controlled conditions.

Some grass root materials have been collected in Brazil, Florida, Wisconsin, Maryland, Ecuador, Venezuela and West Africa. Root-associated nitrogen-fixing bacteria were isolated from these samples. The bacteria appear to be widespread and adapted to a wide variety of soil conditions. These new isolations of Spirillum from several tropical countries will be tested against grass genotypes to determine effectiveness. As new bacterial introductions and new plant genotypes are obtained, they will likewise be screened for effective associations. Preliminary results have indicated genotypic specificity between grass and bacteria. The most effective combinations remain to be determined. Studies under this objective must be coordinated with those under other objectives.

a) Work Phases (may be conducted simultaneously)

1) Collect and evaluate nitrogen-fixing bacteria isolated from grass root systems.

a) Isolate from grass roots promising N-fixing bacteria.
b) Measure growth rates and N_2-fixing ability.
c) Evaluate infectivity or association with grass roots and determine N-fixation by the associations.
d) Reisolate bacteria from successful effective plant associations.

2) Characterize isolates showing promise of significant associative N-fixation in ways that make it possible to identify them in mixed cultures.

a. Morphological
b. Serological
c. Physiological and biochemical
d. Other techniques as needed.

3) Determine effect of environmental conditions and nutrition on N-fixing bacteria, including the following:

a) Temperature requirements
b) Nutrient requirements
c) Oxygen requirements.

4) Produce mass cultures of selected bacteria for use in inoculation studies in plant studies in greenhouse and field.

a) Specific growth conditions.
b) Viability and population of inoculants

5) Determine the ability of N-fixing bacteria to survive and colonize soils of different characteristics, including these conditions.

a) Presence or absence of host plants
b) Acid and alkaline soils
c) Low and high organic matter
d) Different clay minerals

6) Investigate the genetic stability of the selected bacteria.

b) Resources Required:

1) one faculty microbiologist, technologist and extra labor.
2) supplies for operating microbiological laboratory.and equipment to supplement that currently available.
3) travel and allowance, both domestic and foreign.
4) data processing and publications.

2. Identify and define plant and bacteria characteristics critical for effective association and N_2-fixation and to identify more effective plant genotypes for N_2-fixation.

It is important and essential to identify plant characteristics important in bacteria grass-plant association and N_2-fixation. These factors have never been evaluated. Plant genotype specificity has been implied but not proven. Plant factors are probably more important than most environment factors and could be the cause of high failure rates in establishing effective N_2 fixation. Several aspects of these studies will be undertaken simultaneously.

a) Work Phases (may be conducted simultaneously)

1) Comparison of efficient vs poor N_2-fixing genotypes within the same grass species.

a. biochemical comparisons
b. physiological comparisons
c. morphological comparisons
d. microbiological comparisons

2) Study ability of plants (genotypes) to form N-fixing bacterial associations (infection).

3) Screen for more efficient bacteria-plant combinations.

4) Study basic mechanisms of infection and N_2-fixation.

5) Use genetic manipulation to transfer desired characteristics in both plants and bacteria.

b) Resources required.

This objective will require more intensive effort in the laboratory, but some in the field. Laboratory requirements will be extensive, including controlled environment plant growth facilities, instrumentation and supplies for a wide array of biochemical, physiological, microbiological and cytological studies.

1) Technologists and extra labor.

2) Supplies for greenhouse, laboratory studies and field studies, equipment to supplement that currently available.

3) Analyses of nitrogen, acetylene-ethylene, ^{15}N, etc.

4) Rental of field equipment.

5) Travel and allowance - both foreign and domestic.

6) Data processing and publication

3. Define critical environmental conditions for effective N fixation for each association.

Evaluation of environmental factors is necessary to more effectively study and evaluate N_2-fixation by grass systems. A key to understanding limitations to associative biological nigrogen fixation is to study the variables under controlled conditions. When the factors are better understood the studies will be extended to field situations with inoculation to evaluate soil and climate effects.

Accepted experimental techniques will be used including replication and statistical analysis. Statisticians at the University of Florida are readily available for consulting without being assigned to the project. Results will be evaluated using both the Wang calculator (programmed) or a computer. Analyses will include acetylene reduction, (in situ where possible), yield, nitrogen balance and nitrogen balance and nitrogen content of forage produced.

Investigations will involve different crop species and bacterial strains, therefore, evaluation of each system will be needed. Sub-contracts are planned to extend tests to different environments.

a) Work Phases

1) Using data from work under objectives 1 and 2, optimum N_2-fixation will be determined with:

(a) best grass genotypes available.

(b) optimum growth conditions, including 31°C soil temperature, control of pO_2, adequate nutrition, especially Mo, controlled use of N, optimal light and other factors.

2) Evaluation of environmental factors:

(a) Use effective system and vary each factor.

(b) Use approved experimental designs with adequate replication followed by statistical analyses.

(c) Study factors in combination to determine interactions.

(a) Oxygen levels
(b) Temperature and light
(c) Mineral nutrition
(d) Inoculation

3) Develop effective methods of inoculating plants of various species with various microrganisms.

(a) Develop more efficient methods of placement.
(b) Develop ways to stabilize inoculum bacterial populations.

4) Study environmental factors in the field.

(a) Study effects of soil types at various locations.
(b) Study climatic effects.

To determine changes in bacterial populations soil ecological techniques will be employed, such as fluorescent antibody techniques of Schmidt.

The investigation will involve different crop species and bacterial strains, therefore, evaluation of each system will be needed.

(b) Resources Required

(1) Technicians and extra labor.
(2) Supplies for greenhouse and growth chamber facilities and equipment to extend range of temperature, light intensity, oxygen levels, etc.
(3) Analyses of total N, bacterial populations, using fluorescent antibodies, plant tissues for mineral nutrients, etc.

(4) Supplies for field work, equipment to monitor environmental variables and equipment rental

(5) Data processing and publications.

4. Measure amount of nitrogen fixed and the value of fixed nitrogen in replacing fertilizer N.

Studies under objectives 1, 2, and 3 are aimed at providing biological material and the knowledge required for successful, dependable grass N-fixing systems in the field.

Some of the ecological factors are not easily manipulated on the field scale. The major objective is to provide economic and important nitrogen inputs to the grass crop production and contribute significantly to the world's food supply. To evaluate the nitrogen contribution to the grass crop will require major field and laboratory work.

Data from practical situations are needed to evaluate the various systems that have been developed. Techniques will have to be evaluated and modified to accurately determine nitrogen inputs from the grass-bacteria association.

a) Work Phases

1) Use cost efficient, but accurate methods for monitoring nitrogen fixed in greenhouse and field studies - need to modify highly criticized "Dobereiner technique." Techniques will include:

a) Yield differences
b) N balance methods
c) *In situ* acetylene reduction
d) Inoculated vs not inoculated comparisons (yield of dry matter and N)

2) Evaluate best crop-bacteria associations by field testing at several locations. When dependable associations are developed, the N contribution will be measured in different field situations with a standarized design using fertilizer N rates to determine fertilizer equivalent, and to determine the consistency of the association under different field situations.

3) Measure plant development and bacteria populations after inoculation.

b) Resources required.

Field work is essential in studying these systems because the practical value of the associations must be determined in the field. The large number of replications required (N_2-fixation increases variability), the difficulty of accurately measuring N_2 fixation and its effect on productivity or N fertilizer replacement, and inoculated trials will require considerable field area and numerous plots.

1) Technicians and extra labor.

2) Equipment rental for crop culture, inoculation, environmental monitoring, transport of materials and labor.

3) Costs of plant and bacteria analyses, for example, nitrogen, including ^{15}N, acetylene reduction.

4) Travel

5) Data processing and publications.

(9) Overall Cost Estimate by outputs: Nitrogen Fixation by Grass-Bacteria Associations (See Attachment).

(10) Time Phased Work Plan and Expected Outputs (See Attachment).

(11) GENERAL APPRAISAL

This project proposal has been discussed with Dr. Plucknett, Chief, Soil and Water Management Division and Dr. Baird, Research Director, TA/AGR. On 18 December 1975, an in-house technical review with agricultural technicians from the bureaus and a meeting with Dr. Long and TA/RIG were held.

1) The concensus was that nitrogen fixation by grass-bacteria systems is a vital topic in which TA/AGR should be involved. The research purposes were worthy, but the objectives and the project statement needed extensive revision. The revisions have been made.

Other issues discussed were as follows:

2) Why not contract with Dr. Dobereiner and her staff in Brasil?

AID has a task order under consideration to support the Brasil program by providing certain equipment and for the exchange of scientists through the National Academy of Sciences. Dr. Dobereiner has said that she has adequate funding.

3) Wouldn't AID funds be more appropriately spent to piggyback on U.S. research?

In this project AID will be utilizing capabilities and facilities brought together with state and federal (USDA) funds. AID is asked to participate in this work, to share in ongoing and special costs so this vital topic can be investigated more quickly and completely with consideration of tropical conditions. The University of Florida and USDA are also making considerable financial contributions.

4) Why should AID finance this project until such time as the application of nitrogen fixing grass-bacteria systems can be tested under farming systems in the LDC's?

The value of nitrogen fixation by grass-bacteria systems has such great potential that AID should support research to determine the feasibility of the system as quickly as possible. AID can not afford to wait until some one else develops a system for the family farmer in the tropics. This research is urgently needed to determine whether a dependable grass-bacteria system can be established.

5) Does University of Florida have the capability to conduct this research?

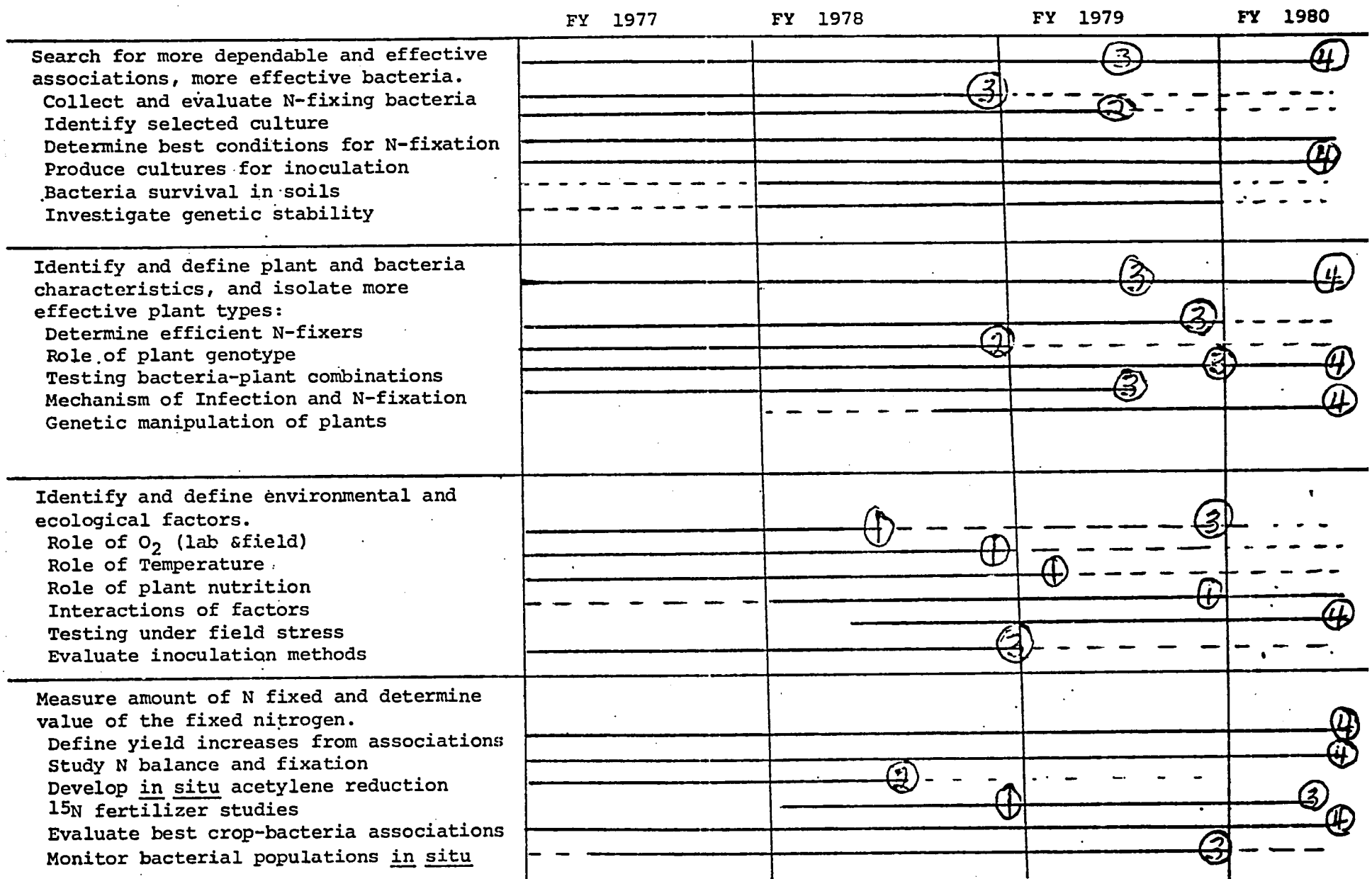
During the past 2 years, University of Florida has moved very rapidly, using their own financial resources, USDA support and a minigrant from AID begining about October, 1975 to build capability and facilities. Because of their strong foundation in tropical agriculture and their commitment, they now have a team capable of undertaking this research in FY 1977.

XI. C. Environmental Impact

The ultimate aim of this project is to increase food availability to people of the LDC's. To do this, it is hoped to increase biological nitrogen fixation and reduce the need for adding fertilizer nitrogen, conserving fossil fuels and reducing potential soil and water pollution.

Outputs of this project should not adversely affect people or the environment. The ultimate benefits of this work would be most beneficial to the small farmer who can not afford fertilizer nitrogen.

TIME PHASED WORK PLAN AND EXPECTED OUTPUTS*



*Circled numbers indicated expected outputs; see following page for explanation.

The research planned under the various objectives is scheduled to interact and is coordinated to provide information and biological materials for research on other objectives. Some of the research studies, as shown by lines across all 4 years, is expected to continue beyond 3 years if dependable and effective associations are developed as new and better plant and bacteria genotypes are found and/or developed.

Major expected outputs are noted by the circles. The following key defines these outputs:

(1) controlled environmental studies complete; field observations will continue.

(2) research expected to be completed.

(3) major efforts completed; minor follow-up research anticipated.

(4) research expected to be continuous.

PROPOSED BUDGET BY INPUTS: NITROGEN FIXATION BY GRASS - BACTERIA ASSOCIATIONS

	First Two Fiscal Years				2 Year Work Plan Subtotal		To Completion		Total Est'd Life of Project Research Cost
	Man Mos FY	Est'd Cost 000 1977	Man Mos FY	Est'd Cost 000 1978	Man Mos	Est'd Cost 000	FY 1979 Est'd Cost	FY 1980 Est'd Cost	Est'd Cost
Inputs									
Salaries	160	112	180	120	340	232			
Consultants	4	2	4	2		4			
Fringe Benefits	80	12	90	12	170	24			
Overhead	160	63	180	68	340	131			
Travel (Common Carrier)		4		6		10			
Allowance (Per Diem)		3		5		8			
Other Direct Costs		8		9		17			
Equip. Vehicles Material & Supplies		43		35		78			
Publications		1		1		2			
Subcontracts		20		35		55			
Total Costs by Inputs	160	268	180	293	340	561	310	320	1191

BUDGETARY SYPPLEMENT: PROPOSED CONTRIBUTIONS BY VARIOUS AGENCIES TO RESEARCH PROJECT ON ASSOCIATIVE NITROGEN FIXATION - 2 YEAR TOTAL (FY 77 AND FY 78)

	Univ. of Florida 000	USDA 000	AID 000	Total Project Costs 000
Salaries	130	100	236	466
Fringe Benefits	22	13	24	59
Overhead	75	56	131	262
Travel & Trans.	4	2	10	16
Allowance	2	1	8	11
Other Direct Costs	10	5	17	32
Equip. Vehicles, Land) Materials, supplies)	42	27	78	147
Publications, reports	3	2	2	7
Subcontracts	-	-	55	55
Total-2 yr period	288	206	561	1055

OVERALL COST ESTIMATE BY OUTPUTS: NITROGEN FIXATION BY GRASS-BACTERIA ASSOCIATIONS

	FY 1977 Man Mos	FY 1977 Est'd Cost 000	FY 1978 Man Mos	FY 1978 Est'd Cost 000	2 Year Budget Man Mos	2 Year Budget Est'd Cost 000	FY 1979 Est'd Cost 000	FY 1980 Est'd Cost 000	Total Est. Life-of-Project Research Cost Est'd Cost
Outputs									
Research Obj. #1	46	88	51	96	97	184			382.
Search for more dependable and effective associations									
Research Obj. #2	37	53	42	54	79	107			210.
Identify and define plant and bacteria factors									
Research Obj. #3	31	49	36	51	67	100			169.
Identify and define environmental and ecological factors									
Research Obj. #4	46	58	51	57	97	115			296.
Measure amount of N fixed and its value.									
Sub-total w/o sub-cont.		248		258		506			1,057.
Sub-contracts		20		35		55			135.
Total Cost by Outputs (including sub-contracts)	160	269	180	293	336	561	310	320	1,191

AID 1020-28 (1-72)

PROJECT DESIGN SUMMARY
LOGICAL FRAMEWORK

Life of Project:
From FY 77 to FY 8
Total U. S. Funding $2,200,0
Date Prepared:

Project Title & Number: NITROGEN FIXATION BY ASSOCIATIVE GRASS-BACTERIA SYSTEMS

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Program or Sector Goal: The broader objective to which this project contributes: Increase food production and improve quality of life of small farmers through better soil and water management.	Measures of Goal Achievement: Measurement of biological nitrogen fixation by associative grass-bacteria systems. Increased food production by grasses, including cereals, by increased biological nitrogen fixation	Data from experimental field plots given in reports and publications.	Assumptions for achieving goal targets That grass-bacteria nitrogen-fixing association can lead t increased food production and increased income on family farms.
Project Purpose: To critically study tropical grass-bacteria associative nitrogen fixation. To evaluate its potential for world-wide use, especially in LDCs. To isolate, characterize and evaluate the N-fixing ability of bacteria associated with grass roots. To determine if these systems can be managed and placed into farming practice on a practical basis.	Conditions that will indicate purpose has been achieved: End of project status. Critical data that definitively determine the amount of N-fixed by associative grass-bacteria associations. If significant N-fixation occurs development of practical systems for use in farming.	1. Annual reports from investigators 2. Adoption of systems by some farmers.	Assumptions for achieving purpose: That grass-bacteria nitrogen - fixing association is amen able to manipulation and dev -opment.
Outputs: 1. An associative grass-bacteria system that consistently fixes appreciable nitrogen. 2. Environmental conditions, grass cultivars and bacteria that improve nitrogen fixation, esp. in the tropics 3. Field inoculation methods. 4. Training of research personnel 5. Linkages with international and U.S. agencies.	Magnitude of Outputs: 1. At least one successful system providing more than 20% of the nitrogen needs. 2. Not directly quantifiable 3. At least one successful inoculation method. 4. At least 10 graduate students, some from LDC's. 5. Linkages with at least 2 international centers, 2 LDC's, USDA, and universities in US and Brasil	1. Reports by contractor; on-site inspection 2. Reports by contractor 3. Reports by contractor 4. Contractor reports. 5. Contractor reports, communications from other agencies.	Assumptions for achieving outputs: 1. The associative system ca provide significant amounts of nitrogen. 2. N-Fixation controlled by genetics and environmen 3. Association can be established by inoculation 4. Personnel available 5. Sufficient interest among various agencies.
Inputs: 1. AID/W provides financial support and project guidance. 2. Contractors provide qualified personnel and major facilities. 3. Cooperation provided by (1) other universities, (2) international centers, (3) LDCs.	Implementation Target (Type and Quantity) 1. AID/W funding at approximately $300,000 per year 2. 162 man months per year from senior staff, 10 to 12 graduate students, and 4 to 6 technicians; adequate laboratory facilities; more than 5 acres field research area. 3. Not directly quantifiable.	1. AID/W records 2. Contractor reports 3. Reports, on-site verification.	Assumptions for providing inputs: 1. Appropriate AID/W funding will be available. 2. Contractor has adequate personnel and facilities available to project and will subcontract to augment environments and expertise. 3. Continued cooperative support from other agencies, especially other universities, USDA and international centers