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PD-AAD-240-B1

PROJECT EVALUATION SUMMARY

(Submit to MO/PAV after each project evaluation)

1. Mission or AID/W Office Name DS/Agriculture/TSWM Division	2. Project Number 931-1004A 8p

Project Title

Nitrogen Fixation by Associative Grass-Bacteria Systems

3. Key project dates (fiscal years) a. Project Agreement Signed Fy 77 Feb. 1, 1977	b. Final Obligation Fy 79 12/31/78	c. Final input delivered Fy 79	5. Total U.S. fund life of project $ 232,950

6. Evaluation number as listed in Eval. Schedule N/A	7. Period covered by this evaluation FROM: 2/77 Month/year	TO: 5/78 Month/year	8. Date of this Evaluation Review 5/26/78 month/day/year

9. Action Decisions Reached at Evaluation Review, including items needing further study (Note--This list does not constitute an action request to AID/W. Use telegrams, airgram, SPARS, etc., for action)	10. Officer or Unit responsible for follow-up	11. Date action to be complete
1. With additional funding, extend the contract for 3 years to determine feasibility of the associative nitrogen fixation by grasses in tropical and sub-tropical agriculture	Joint: University/ Florida/DS/AGR	11/78

12. Signatures:

Project Officer	Mission or AID/W Office Direc
Signature: Lloyd R. Frederick	Signature: [illegible]
Typed name: Dr. Lloyd R. Frederick, DS/AGR/TSWM	Typed name: Leon F. Hesser Director DS/[illegible]

Review and Evaluation

of

Contract AID/ta-c-1376

Project Title: Nitrogen Fixation by Associative Grass-Bacteria Systems

Project With: The University of Florida

Date of Review: May 25 and 27, 1978

Evaluation Panel:

Dr. R. H. Burris, Univ. of Wisconsin	8/2/78 Date
Dr. T. J. Cunha, Calif. State Polytechnic Univ.	8/9/78 Date
Dr. W. L. Fuglie, AFR/RA/AID	9/1/78 Date
Dr. C. C. Black, Univ. of Georgia (Panel Chairman)	7/25/78 Date
Dr. L. Frederick, AID/DS/AGR (Project Officer)	9/11/78 Date

PROJECT EVALUATION REPORT - NARRATIVE

Project: Nitrogen Fixation by Associative Grass-Bacteria Systems
with
the University of Florida

1. SUMMARY

The Florida group has made a systematic attack on the problem of grass/bacteria associative symbiosis for biological nitrogen fixation. They have established that the bacteria are attracted to grass root hairs and can invade by way of root hairs, points of damage on the roots, and at the juncture of lateral roots. Field tests have demonstrated a 15-20% increase in dry matter and total nitrogen for two growing seasons with pearl millet and Panicum maximum. Other associations have not given this great an increase. Increases are obtained only when sandy soils low in nitrogen are supplemented with 40-60 kilograms of available nitrogen per hectare. Unfortunately, there is some inconsistency in the results, and field tests in the summer of 1977 showed very low increases, apparently because of a drought. The bacteriologists have succeeded in developing a doubly-marked mutant for field studies to establish that the specific Azospirillum added to the soil is the one recovered in association with the plant. Using a fluorescent antibody labeling technique, the group has visualized the bacteria associated with plant tissues. This labeled antibody has been helpful in understanding how invasion occurs, where organisms reside on the plant, and in beginning to study the possible association of several bacteria during nitrogen fixation.

Tests of 15_N dilution to measure the biological nitrogen-fixation are incomplete, but it is hoped this will give a reliable index of the contribution of biological nitrogen fixation to the growth of grasses. Tests with soil cores demonstrated a linear acetylene reduction, whereas tests with excised roots which had been washed and preincubated at a low pO2 gave non-linear rates of acetylene reduction with time. This suggests that the soil cores are a more reliable index of fixation in the field. Of particular interest is the observation that 6% O_2 over the soil cores supports a substantially higher rate of acetylene reduction than does 21% O_2. It is apparent that the research project embraces a complex biological problem and that a continuing effort will be necessary to bring the system to a point where its application can be properly evaluated. We suggest a 3 year extension and a review of progress in three years.

2. EVALUATION METHODOLOGY

A special evaluation was conducted in Gainesville, Florida by a five-member review team consisting of Dr. R.H. Burris, University of Wisconsin, Biochemistry; Dr. T.J. Cunha, Dean, California State Polytechnic University, Pomona, Nutritionist/Animal Scientist; Dr. W.L Fuglie, AFR/RA/AID Washington and Agricultural Economics; and Dr. Dr. C. C. Black, of the University of Georgia, Biochemistry,

who served as chairman of the group. Dr. L. Frederick, AID/DS/AGR Project Officer, assisted. Oral reports were presented for a day by Florida research personnel including:

Dr. S.H. West
Assistant Dean for Research, IFAS

Dr. Rex L. Smith
Agronomy Department

Dr. Stanley C. Schank
Agronomy Department

Dr. Ken H. Quesenberry
Agronomy Department

Dr. Murray H. Gaskins
USDA, SEA/FR

Ms. Chad Garcia
Soil Science Department

Mr. James R. Milam
Microbiology & Cell Science Department

Dr. David A. Zuberer
Microbiology & Cell Science Department

Dr. Dennis E. Duggan
Microbiology & Cell Science Department

Drs. Hubbell and Tyler, project investigators, were absent and Chad Garcia and Jim Milam gave the results of their work. Drs. Vasil and Gamble, co-operators, did not give a report. Dr. Paul Smith, Chairman of the Microbiology and Cell Science Department, also gave a brief report about the interests of his department in cell recognition for a specific binding site.

A copy of the Annual Report and the contract was furnished to the review team prior to the site visit. Copies of reprints or pre-prints were furnished to the committee. The review committee considered the scope of work as given in the April 3, 1978 AID memorandum of Hesser to Simpson and to the additional considerations raised in the RAC minutes of the March 22-23, 1976 meeting.

On the second day the review team prepared their evaluation. Then they met with the Florida record group for about two hours to discuss the evaluation of research accomplishments and also to make suggestions about future research.

3. Documents to be revised. This evaluation may result in an extension research project proposal from the University of Florida.

4. Evaluation findings about EXTERNAL FACTORS. The University of Florida and the USDA support this work.. The University of Florida administration provides a large input in faculty, staff, and other resources in this project. USDA support apparently continues at about the same level as previously (about $100,000 per year).

International research interest in grass/nitrogen-fixation associations has lessened somewhat because of the complexity of the problem and the difficulty in consistently obtaining positive experimental results. However, the basic assumptions in the project design are still valid.

5. Evaluation findings about GOAL/SUBGOAL.

Program Goal: To increase yield and protein of grass crops through biological nitrogen fixation.

Plant yield, dry matter and protein, increases (15 to 20%) have been obtained in field experiments. However, these have not been obtained consistently in year-to-year experiments. These yield results, however, are still the most encouraging field data in the literature (Science 193, 1003-5, 1976). It is now apparent that achievement of the goals specified in the project within two years is unrealistic and it will take much longer for Florida scientists and others to resolve this complex biological problem. At least 3 additional years are required to determine whether this system has real promise in agricultural crop production. Then 5 to 10 years will be needed to implement this system in LDC agricultural systems.

6. Evaluation findings about PURPOSE.

Project Purpose: To develop a dependable associative grass-bacteria system that fixes significant amounts of the nitrogen used by the plant.

Two grass/bacterial systems (pearl millet and guinea grass/*Azospirillum*) have been shown to increase dry matter and total protein (15 to 20%) in two years of trials. This was not reproducible in a third year, possibly due to a drastic drought. Several other associations have been obtained but again they were not reproducible in year-to-year studies. Although 20% yield increases have been obtained, evidence of increased nitrogen-fixation as the sole contributing factor has not been established.

7. Evaluation findings about OUTPUTS AND INPUTS.

a. Appropriate grass plant genotypes. Successful grass associations with appropriate bacteria have been demonstrated with two grass plant genotypes; but with many other genotypes that have been examined, the increase in nitrogen and total biomass yield in response to bacterial inoculation has been less than 20% and rather inconsistent.

b. Adapted nitrogen-fixing associative bacteria that can be inoculated successfully.

By demonstrating the association of Azospirillum with the root hairs of specific grasses, it has been possible to show that there is a specificity in the process. The bacteria appear in high concentration on the root hairs, but in much lower concentration on the adjacent root, indicating a specific attracting or binding substance.

c. Environmental conditions required for functioning of the nitrogen-fixing system.

It has been demonstrated with Azospirillum in soil that fixation is very slight at temperatures above 42°C. The experiments also show that the organisms do not survive at -20°C. Experiments during drought years suggest that the organisms do not tolerate dry conditions well. The organism reduced very little acetylene at a p^H below 5.5. They also become inactivated rapidly at p^H above 7.8. One of the more interesting observations is that soil cores, taken to include the root system of grasses, reduce acetylene much more rapidly in the presence of 6% O_2 than in the presence of air. The soil cores showed a linear reduction of acetylene in contrast to an increasing rate of reduction by excised and washed root systems.

d. Tests of functioning systems for a few seasons at several sites.

It has not been possible, with the reduced funding for the project, to investigate the fixation at a number of sites. All tests have been made in the vicinity of Gainesville on sandy soils. Success with increased yield has been verified at a few other stations, although their responses have been quantitatively less than those observed in Gainesville. There have been reports of positive responses in Brazil, the Bahamas, Nebraska and Wisconsin. There has been linkage with other investigators through workshops held during the past two years at Gainesville. A substantial percentage of other persons working in field nitrogen fixation in the United States have attended

these workshops. The Florida scientists have made it a point to attend international meetings in which associative symbiotic systems have been discussed at length, and they have contributed to these symposia. The State of Florida has contributed to the program to a greater extent than was suggested in the project design summary.

8. Evaluation findings about UNPLANNED EFFECTS.

It is surprising that in order to get a response from the associative symbiotic bacteria with a plant it is necessary to have a moderate amount of nitrogen available in the soil. Usually, the best response is obtained when 40 to 60 kilograms of nitrogen per hectare are available. The response of the plant is over and above the response level of nitrogen. The inconsistency of the fixation from year to year has also been somewhat surprising. Although an increase in dry matter and total nitrogen has been achieved with inoculated plants, it has been particularly difficult to establish that this response can be attributed solely to nitrogen fixation, rather than to secondary plant growth responses. These responses have been taken into consideration by the research group in their projected experimental design. Present data indicate that one can depend upon associative symbiosis for only a part of the nitrogen requirements for grasses grown on low nitrogen soils. This limited response suggests the necessity for re-examining environmental factors and for broadening the search for more effective nitrogen-fixing organisms. It is quite possible that mulching may minimize the effects of excessive temperatures and drought and thus extend the useful range of associative symbiosis.

The unexpected effects will increase the time base for achieving success in the application of associative symbiotic nitrogen-fixing systems in grasses to practical agricultural systems.

9. CHANGE in DESIGN or EXECUTION

The major change that emerges from the site visit is the necessity for extending the period of the contract. An extension for 3 years seems reasonable in order to achieve the purpose outlined. Although a 3 year extension is reasonable, there should be no illusion that a system of this complexity can be completely resolved in a 3 year period. This programm must be considered as a high-risk project and one which cannot be translated immediatley into practical application. The visiting group feels strongly that tests should be made in soils other than those characteristic of the Gainesville area. If possible, plots should be set up in tropical areas, either by this group or by collaborators. It may be feasible to establish cooperation with one of the international agricultural research centers to test associative sym-

biosis under tropical conditions. Cooperation with one of the CRSP programs, particularly the one involving sorghums and millets, is another posibility for testing in the tropics. The site visit group suggests that the tests of nitrogen-fixation by ^{15}N dilution be continued, as these constitute an independent method of establishing the direct contribution of nitrogen fixation to the economy of the plant. There should be a continuing emphasis both on the role of the grass cultivar and the bacterial strain in an effort to find associations which are particularly effective in nitrogen fixation.

The site evaluation committee recommends that the funding for this project be increased to at least $200,000 per year. This amount should be further increased to $300,000 per year if substantial collaboration occurs with LDCs. This recommendation is made with the assumption that there will be a continuation of the present contribution from the USDA and the University of Florida.

10. LESSONS LEARNED.

A search should be continued for grass/nitrogen fixation associations and broadened to include a variety of plants and bacteria in addition to the Azospirillum associations. Similar research is underway in other countries and continued research contact should be encouraged. AID, when investing in research, must provide some high-risk opportunities which, if successful, would give a high payoff.

11. SPECIAL COMMENTS or REMARKS.

Due to variability in total yields and to an apparent need to supplement the soil nitrogen level, research results to date are not ready for utilization in LDCs. However, if the above can be rectified, especially on food grains, the probability of farmer adoption might be greatly increased. Farmers who may first utilize the technology would be those already having a suitable soil nitrogen condition, i.e, the first years of production in a bush-fallow farming system, after legume plough down, or after a commercial nitrogen broadcast application.