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EGYPT: BIOLOGICAL NITROGEN FIXATION RESEARCH AND TECHNOLOGY ASSESSMENT

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PREFACE

The study team was pleased to work with scientists and others in Egypt to assess the biological nitrogen fixation (BNF) science and technology in Egypt. The team realized that appropriate use of BNF technology by Egyptian farmers could provide increased amounts of crop production at lower cost, thus benefitting both the farmer and the Egyptian society.

The team was encouraged to find that many Egyptians recognized the potential value of BNF, and that a cadre of well-trained scientists were working on various BNF systems. Research is currently in progress on the *Rhizobium*-legume system, the associative and asymbiotic N_2 fixing bacteria system, the *Azolla*-flooded soils system, the blue-green algae-flooded soils system, and the *Frankia*-*Casuarina* system. It was disappointing to find that the opportunity for farmers to use BNF technology was limited by inadequate supply and ineffective legume inoculants, inappropriate use of nitrogen fertilizer, and underuse of phosphorus fertilizers, inappropriate irrigation and drainage techniques, and lack of proper technology transfer. All of these limitations can be overcome, however, by a coordinated program of research, training, and extension education. Appropriate motivation for distribution and proper use of high quality inoculants must be provided.

The study team was surprised to find that soybeans were being fertilized with 60 to 90 Kg nitrogen per feddan, according to Ministry recommendations, especially since Brazil and U.S. soybean producers rarely use nitrogen fertilizer. Reports indicated that farmers were obtaining increased production with nitrogen applied to other legumes, even berseem and faba beans. This indicates that the current BNF system may be inadequate and that an opportunity exists to improve the BNF system, decrease production costs, and increase production.

This report is aimed at the general assessment of BNF science and technology in Egypt. Any projects to implement these suggestions contained herein should be designed separately, possibly by a joint Egyptian-U.S. team. Specific suggestions are included for the activities and facilities available under the legume and farming systems amendment of the Egyptian Major Cereals Improvement Project (EMCIP), and for activities under the Rice Project. These ongoing projects provide an opportunity for rapid initiation of BNF activities related to EMCIP and rice production. The universities can participate through use of the grants program. Successful completion of these limited activities can increase BNF use in Egypt.

Continuation and expansion of the BNF program, even with relatively small investments, can improve the welfare of all people in Egypt.

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University personnel at Ain Shams, Alexandria, Cairo and Monoufeia were gracious with assisting the team as it visited the various facilities. Faculties and staff at the universities were involved with the team in discussions, program presentations and facilities viewing. We acknowledge this help and we enjoyed the hospitality and helpful attitude displayed although we interrupted their day and appeared excessively "nosey." Dr. A. S. Abdel-Gaffar spent long hours assuring that our visit to Alexandria and the university accomplished our objectives. His hospitality is most appreciated. Special thanks are due to Dr. S. A. Zaky Mahmoud, Ain Shams University, to Dr. Shatla, Monoufeia University, and Dr. Monib and Dr. N. A. Hegazi, University of Cairo.

Staff members of USAID/Cairo contributed significantly to the success of our mission. Mr. John Rogers helped generously throughout the team's visit.

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INTRODUCTION

With a 2.5 - 3.5% annual population growth and a limited area of suitable crop land, Egypt faces a serious problem in food production. Legumes are a high quality livestock forage and an excellent protein source for human consumption. Nitrogen fertilizer inputs into legumes should be unnecessary. Egypt presently has large crop areas devoted to legumes, particularly faba beans and berseem clover, but also lentils, cowpeas, lupines and green peas. Yet soybean, chickpea, and peanut production are increasing. To improve and increase protein production for human consumption and provide quality livestock forage without further dependence on nitrogen fertilizer, legume production should be increased.

Biological nitrogen fixation is the key to greater legume production without substantial inputs of nitrogen. Much is already known about the legume N_2 fixation system and can be immediately applied. However, more information is needed to address the problems which limit BNF in Egypt.

Successful inoculation of all the soybeans planted in Egypt could save the farmers L.E. 900,000 each year in nitrogen fertilizer, assuming the use of 60 Kg/Fd of fertilizer nitrogen on 100,000 feddans of soybeans, and a subsidized price of 15 pt. per Kg of fertilizer nitrogen. The real cost to Egypt would probably be at least twice this amount.

Non-legumes, in association with other biological N_2 fixing systems, may also benefit from increased emphasis on biologically fixed nitrogen. Presently, less is known about these systems than the legume system and considerably more research is needed. Yet greater N_2 fixation by non-legume systems could further reduce Egypt's nitrogen fertilizer inputs.

USAID recognized the importance of BNF in increasing food and forage production and asked for an assessment of past, present, and future research. A team of five scientists with diverse backgrounds, experience, and expertise was selected. This report summarizes what was learned about past and present BNF research and suggests a program that could be initiated now and further developed in the future. Additionally, the team has suggested facilities and activities within the Egypt Major Cereals Improvement Project with respect to Rhizobium-legume N_2 fixation research and inoculant technology, and an assessment of the use of Azolla and blue-green algae in rice production for consideration by the Rice Project.

SUMMARY OF SUGGESTIONS/RECOMMENDATIONS

1. Egypt has a nucleus of well-trained scientists who have experience in BNF studies. Increased financial support for research operations is needed to encourage high quality field research in order to implement BNF technology that has been developed and to work on problems that limit the use of BNF technology by farmers. The Ministry of Agriculture and Food Security must support a continued and expanded major effort to conduct BNF research and to improve and implement BNF technology.

2. Transfer of BNF technology to the farmer by extension or outreach activities is a major need in the BNF program. Useful and known BNF technology must be incorporated into a "package program" of practices and presented to the farmer by field demonstration and other techniques. The benefits for the farmer in the "package program" will improve efficient crop production.

3. Extension or outreach activities should be designed and implemented by a team including research and extension scientists. The team carries out the educational program that enables the farmer to incorporate BNF technology into his farming system. The team also identifies problems that limit the use of BNF technology by the farmer and demands that research be conducted to solve these problems.

4. Egyptian universities should provide all undergraduate training for BNF scientists and agriculturalists. Graduate training should continue at universities in several countries, but more training in field work is necessary. Post graduate training and scientist exchange with the U.S. and other foreign institutions is essential for professional enhancement and interchange of ideas and techniques.

5. A coordinated research program in BNF for Egyptian agriculture should be established and should utilize the Agricultural Research Center, the National Research Center, and those universities with capable staff trained and interested in BNF research. A small committee of BNF scientists with rotating membership could provide guidance to Egyptian research in BNF. A competitive grants program for BNF research could help to assure university scientist participation and could focus work on relevant problems. Field research on the Rhizobium-legume system should be emphasized

with smaller but significant efforts on the Associative N_2 fixing bacteria system for cereals, the Azolla and blue-green algae systems for rice, and the Frankia-root nodulated system for Casuarina.

6. Cooperative efforts in research/outreach BNF activities between U.S. and Egyptian institutions should be encouraged. Priority can be given to such arrangements in the EMCIP grants program. The exchange of scientists should continue, and the use of Title 12 funds for this purpose is commended.

7. High quality inoculant should be imported immediately. Inoculants presently produced in the Jiffy peat pellets are ineffective and production should be discontinued. Extension agents and farmers should be trained in proper handling and application of legume inoculants. An urgent need exists to use proper inoculation for soybeans to get optimum yields with the use of little or no nitrogen fertilizer. As demand for inoculant develops, a commercial company could be established to provide high quality inoculant produced in Egypt.

8. The Ministry should also provide quality assurance for inoculants sold and used in Egypt. Development of an Egyptian Inoculant Research and Control Service based on the Australian model should be considered. Quality assurance work will require new or remodeled facilities including clean space for year-round plant growth facilities necessary for testing inoculants and evaluating strain effectiveness.

9. When a comprehensive, coordinated Egyptian BNF program is designed and supported by the Egyptian scientists and authorities, support of the program would be a worthwhile project for an outside agency. In the meantime, appropriate activities can be initiated under EMCIP and the Rice Project.

10. EMCIP wisely included a soil microbiology laboratory in their plans to assure that the contribution of BNF to production of legumes and in farming systems would be improved by use of appropriate BNF technology. A preliminary laboratory design including the essential plant growth chamber together with a list of equipment needed is included in this report. Research activities included the urgent need to establish reliable methods of using high quality inoculants, especially for introduced legumes such as soybeans and all legumes in new lands, determining the need-to-inoculate for each legume grown in Egypt with

an evaluation of its fertilizer, and to evaluate new cultivars for efficiency with selected strains of Rhizobium.

11. An assessment of the potential for BNF technology, including Azolla, blue-green algae, and legume intercrops, should be conducted by the Rice Project. Appropriate BNF research should be included in this project and coordination with related programs in Egypt is recommended.

12. The team appreciates the cooperation and valuable assistance provided by our Egyptian hosts. We recognize that our brief visit may have lead to misconceptions and inaccurate representation of some aspects of BNF science and technology. We trust this report will be read with understanding of our problems, and we hope that those suggestions/recommendations that have merit will be implemented with all due haste.

METHODOLOGY for THE STUDY

Interest in additional cooperative studies in BNF between Egyptian and U.S. institutions was generated during a seminar presentation in Cairo by Dr. S. H. West in January 1980. Discussions continued by correspondence and through another visit to the Egyptian Ministry of Agriculture in February 1981. At that time a proposal to include a number of Egyptian universities, the Egyptian Ministry of Agriculture and the Universities of Florida, Illinois, and Montana State University was approved by representatives from most of the institutions. This proposal included a visit to Egypt by a team of experts from the USA to study the research and extension needs in BNF. During that visit USAID/Cairo approved support for the team study.

The team visited various institutions concerned with BNF in Egypt. Personnel involved in N_2 fixation were interviewed, facilities and programs in progress were observed at Ain Shams University, Alexandria University, Cairo University, Monoufeia University, Agricultural Research Center of the Ministry of Agriculture at Giza and at Sakha.

Information was obtained on previous research and results, current program content and progress, personnel training and research interests. Data were collected on crop acreage, yields, practices and attitudes.

The study team was provided reports, theses, and lists of publications by the Agricultural Research Center and the universities. Dr. Roughley's report of May 1976 and the legume and farming systems amendment to the Egypt Major Cereal Improvement Project were also very useful.

A few farms were visited and the operators were interviewed concerning practices, yields and views of need. Field experiments in progress at all the visited institutions were also examined. Samples were taken to determine degree of nodulation and a few nodules taken for isolation.

People engaged in agricultural enterprises in the private sector were interviewed. Their views on agricultural production practices, interactions with the government sector were collected.

Discussions were held with personnel from the EMCIP. Their progress, procedures, views and suggestions are incorporated.

Finally the team assessments, conclusions and recommendations were compiled in a report to USAID/Cairo. Copies were distributed to all universities, agencies and institutions with concern in N_2 fixation.

The team only had 11 days to investigate the BNF work and its role in Egyptian agriculture. Many omissions may have occurred and misconceptions probably exist that would be corrected with further study. We beg the indulgence of our Egyptian colleagues and trust that more time and greater contact will lead to better understanding and enable us to be of greater service and to derive mutual benefits. It is indeed a privilege to be able to work with Egyptians to help them make their country a better place in which to live for Egyptians.

BACKGROUND

Past BNF research in Egypt is extensive. Sources (literature) for previous research efforts was provided by the universities, the Agricultural Research Center, and several individuals. One Egyptian scientist indicated that 800 publications might exist concerning BNF research in Egypt. The team did not find nearly this number of articles, but undoubtedly many were missed. We were given theses at several institutions, but many pertinent theses were presumably not located. Unfortunately the team was not able to visit Assiut, Zagazig, Menia and other institutions.

Most of the articles were found in abstract and title summaries, proceedings abstracts, and from individual summaries. Any scientist contemplating BNF research in Egypt should consult the abstract and title summaries (1, 2, 3) before starting the research. The I.B.P. reference was particularly useful concerning BNF research through 1972. A new bibliography for the period 1973-1981 would be very useful.

Over 225 abstracts, titles, theses, and reports concerning BNF were found. A quick subject review of these references is listed in the table below.

Summary of Egyptian BNF research publications according to Systems studied (1923 to 1980)

BNF System	Total BNF Research %	Within Each System (%)	
		Basic 1/	Applied 2/
Rhizobium-legume	47	56	44
Associative-asymbiotic	37	67	33
Blue-green algae	15	78	22
Azolla	0	0	0
Frankia and Other	1	100	0
	100		

(refer to following page for footnotes 1/ and 2/)

No publications were found concerning the Azolla BNF system. However, the team did view Azolla research at the Agricultural Research Center in Giza indicating that research is underway in this area and publications should be forthcoming.

Only three references concerning the Frankia BNF system were found. All three references were recent (1979-1981) and were from Alexandria University (Department of Soil and Water Science).

Many references pertinent to the blue-green algae BNF system were found. Most of the research was considered basic (78%). Many of the researchers were not agriculturists or remotely related to agriculture. Practical application of this research will be difficult. Only a few articles concerned field research and were mainly surveys. Several universities and the Agricultural Research Center in Giza were involved in this research.

Many universities were active in associative-asymbiotic BNF research. Most of the research was basic (67%), not applied. Several field and pot experiments were found that indicated the associative-asymbiotic system may have potential in Egypt. Cairo University has been particularly active, especially within the last 10 years. Data presented indicated significant yield responses to inoculation. Considerable literature in this area also concerned ecology and surveys of various environments. Cairo and Alexandria had active programs at desert sites. Most of the literature and current research dealt with Azotobacter and Azospirillum.

The Rhizobium-legume BNF system dominated the literature. Most institutions had some research in this area. Again, the research emphasis was basic, not applied.

Much of the applied research had little direct relevance to solving field inoculation problems. Inoculation field trials, nitrogen fertilization trials, nitrogen balance studies, and N_2 fixation management studies were reported, but did not dominate the literature. Strain trials were conducted primarily under greenhouse and laboratory conditions. Several surveys concerning legume nodulation were noted but were limited in scope.

1/ Basic studies usually involved pure culture in laboratory or greenhouse conditions.
2/ Applied studies usually involved crop plants and microbes in greenhouse or field conditions.

(footnotes apply to previous page)

Articles on carrier and inoculant production were noted, but little concentrated or coordinated effort was found.

Much of the Rhizobium-legume literature dealt with strain studies, pesticide effects, antagonistic effects of other soil microorganisms, and various physical, chemical, and soil factors affecting legume nodulation and nitrogen fixation.

Recently some of the research effort has changed. Although few literature references were found, our visits indicated a shift to more applied and field inoculation work. Montana State University has had a very positive effect on field research at Alexandria. International inoculation trials are being conducted by the universities and the Ministry of Agriculture. The EMCIP program is emphasizing field work, training experience and technology transfer.

A partial inventory of scientists currently active in BNF research in Egypt is given below.

I. Ministry of Agriculture, Agricultural Research Center
Institute of Soils and Water Research
Dept. of Agricultural Microbiology

	Giza		Sakha	
Principal Investigators 2	Present	on leave	Present	on leave
a. Rhizobium-legume				
Researchers (Ph.D)	2	1	2	-
Research Assistants (M.Sc.)	4	1	5	1
Research Assistants (B.Sc.)	3	-	2	-
b. Blue-green algae for paddy				
Researchers (Ph.D.)	2	-	-	-
Research Assistants (M.Sc.)	-	-	1	-
Research Assistants (B.Sc.)	3	-	1	-
c. Azolla				
Researchers (Ph.D.)	2	-	-	-
Research Assistants (M.Sc.)	-	-	-	-
Research Assistants (B.Sc.)	1	-	-	-
d. Associative-Asymbiotic (Azotobacter and Azospirillum)				
Researchers (Ph.D.)	-	-	-	-
Research Assistants (M.Sc.)	3	-	-	-
Research Assistants (B.Sc.)	1	-	-	-
e. Production of Inoculants				
Rhizobium (Ph.D.)	-	-	2	-
(B.Sc.)	-	-	7	-
Laborers	-	-	10	-

I. Ministry of Agriculture, Agricultural Research Center
Institute of Soils and Water Research
Dept. of Agricultural Microbiology (cont')

	Giza		Sakha	
	Present	on leave	Present	on leave
e. Production of Inoculants				
Blue-green algae (Ph.D.)	2	-	-	-
(B.Sc.)	4	-	-	-
Laborers	4	-	-	-

	Present	on leave
II. Alexandria University		
Principal Investigators 3		
a. Rhizobium-legume		
Researchers (Ph.D.) (part time)	1	1
Research Assistant (M.Sc.)	1	
b. Blue-green algae	-	
c. Azolla	-	
d. Associative-Asymbiotic	-	
e. Production of Inoculants	-	

III. Cairo University	
Principal Investigators 2	
a. Phizobium-legume	
Researchers (Ph.D.) (part time)	2
Research Assistants (M.Sc.)	- (supported by Ministry of Agriculture)
Research Assistants (B.Sc.)	-
b. Blue-green algae	
Researchers (Ph.D.) (part time)	2
Research Assistants (M.Sc.)	-
Research Assistants (B.Sc.)	-
c. Azolla	-
d. Associative-Asymbiotic	
Researchers (Ph.D.)	1
Research Assistants (M.Sc.)	2
Research Assistants (B.Sc.)	1
e. Production of Inoculants	

		Present	on leave
IV.	Ain Shams University		
	Principal Investigators 2		
a.	Rhizobium-legume		
	Researchers (Ph.D.) (part time)	5	
	Research Assistants (M.Sc.)	-	
	Research Assistants (B.Sc.)	2	
b.	Blue-green algae		
	Researchers (Ph.D.) (part time)	2	
	Research Assistants (M.Sc.)	-	
	Research Assistants (B.Sc.)	-	
c.	Azolla	-	
d.	Associative-Asymbiotic		
	Researchers (Ph.D.) (part time)	1	
	Research Assistants (M.Sc.)	-	
	Research Assistants (B.Sc.)	1	
e.	Production of Inoculants	-	
V.	Monoufeia University		
	Researchers (Ph.D.)		1
VI.	Zagazig University		
	Researchers (Ph.D.)		2
VII.	Mansouria University		
	Researchers (Ph.D.)	1	
VIII.	Menia	2	
	Researchers (Ph.D.)	2	
IX.	Assiut		
	Researchers (Ph.D.) Faculty of Agriculture	1	
			1 Faculty of Science

Abstract and Title Summaries

1. I.B.P. (Section PP-Nigrogen Fixation) Bibliographical Report. An annotated Bibliography of Egyptian Work on Biological Nitrogen Fixation published during the period 1946-1972.

   Society of Applied Microbiology and Academy of Scientific Research and Technology, Arab Republic of Egypt. 1973. Copies are available on request from: Society of Applied Microbiology 1 Elhami Street, Tager Building, Garden City, Cairo, Egypt.

Abstract and Title Summaries (cont')

2. Bibliography of Microbiological Research in U.A.R. (1923-1964)
The Egyptian Science Association, Society of
Applied Microbiology. 1965.
1 Elhami Street, Garden City, Cairo, U.A.R. Titles only.

3. Bibliography of Microbiological Research in U.A.R. (1965-1968)
The Egyptian Science Association, Society of
Applied Microbiology. 1970.
1 Elhami Street, Garden City, Cairo, U.A.R. Titles only.

EXTENSION

I. Introduction

Egypt is not capitalizing on the potential of the BNF scientists or those working in related disciplines to make their full contributions to increase farm production and profits and, in general, to promote the agriculture industry. This applies to the Ministry of Agriculture scientists in the Agricultural Research Center and the Extension Service, the Faculties of Agriculture of Egyptian Universities, and to scientists and agriculturists employed in the private sector.

The inability of Egypt to capitalize on the potential of these scientists to make their full contributions to promote the agriculture industry through the introduction of known BNF technologies results primarily from institutional constraints. The principle constraints involve inefficient organization, inadequate budgets, lack of field experience by scientists, and the unavailability of necessary BNF inputs such as viable, effective *Rhizobium* inoculants.

Development of a coordinated cooperative national BNF program could enable Egypt to benefit more quickly from the use of BNF technology in agriculture. The national BNF program could be coordinated by a 3 to 5 person committee of BNF scientists with a rotating chairmanship and each member to serve staggered terms of 3 to 5 years. The coordinating committee could determine needs, develop programs and encourage activities to utilize BNF technology through extension, training and research programs.

II. Statement of Need for BNF Technology Transfer

Since soybeans, peanuts, some other grain legumes and alfalfa are exotic crops in Egypt, the proper strains of *Rhizobium* for effective nodulation and N_2 fixation would not be expected to occur among the indigenous microflora of the soils of Egypt. In addition, a substantial number of feddans are planted to these exotic crops, e.g. Egyptian farmers plant about 100,000 feddans of soybeans annually. Furthermore, the Egyptian Ministry of Agriculture recommends that N fertilizer be applied to soybeans at the rate of 60 Kg N per feddan. These three facts clearly point out the need for the immediate introduction of known *Rhizobium*-legume technology into this sector of Egyptian agriculture. This need for the immediate interjection of known BNF technology into Egyptian agriculture is further substantiated by the fact that Egyptian farmers currently apply N fertilizer to soybeans at rates from 60 to 90 Kg N per feddan and that they regularly make supplemental

N fertilizer applications to faba beans and berseem clover. Poor nodulation observed on plants from some fields of these long-present crops explains these farmers' use of N fertilizer to attempt to achieve higher yields. The successful introduction of appropriate BNF technology could divert most, if not all, of this N fertilizer to non-leguminous crops.

III. Current Extension Activities for BNF Technology Transfer

A. General

As is the case with the transfer of other known technologies or newly developed technologies to farmers, known BNF technology or a "package" of known technologies which includes a BNF component must be viewed as a "product of science" to be "sold" to farmers. For effective technology transfer, a well planned sales campaign must be designed around a saleable "product of science." The campaign must take into consideration the product's characteristics, its modes of action, the requirements needed to insure its distribution to the farmers in an effective form, the absolute need for its proper use by the farmer as well as the profitability of its use and any impact it will have on release of capital to other needs within the total farm management scheme. The latter particularly applies to the total needs of the rural farm families of Egypt.

At the time of this writing Egypt does not have any active campaigns to transfer known BNF technologies to farmers. Previous attempts by the Ministry of Agriculture to produce *Rhizobium* inoculants for legumes and their attempts to distribute them to farmers have not been successful. In spite of this lack of success the inoculants are still being produced and offered for sale to farmers who must bring their requests to the Giza facility; this is an activity of doubtful utility. There appears to be four primary reasons for this lack of success 1) the product (inoculants) was ineffective and therefore not a saleable product (see this report's section on inoculum production), 2) the inoculants were not included in a "package" program which included other agronomic practices such as proper crop variety selection, irrigation water management, seedbed preparation, phosphate fertility, weed control, etc., any one of which, if neglected or improperly done, can lead to the failure of effective inoculants, 3) an effective channel from the scientists down to the farmers does not exist due to institutional regimentation, 4) grossly insufficient funding of this activity by the Ministry of Agriculture, and 5) incomplete understanding of the farmers' constraints, concerns and motivation.

Marketable technologies have not evolved from the *Azolla*, blue-green algae, associative N-fixation systems or *Frankia*-woody

plant symbiosies programs due to the newness of these technologies and the slow research progress largely resulting from the same constraints mentioned above.

B. Ministry of Agriculture

In addition to many of the above constraints, the Ministry of Agriculture's extension program has evolved into an ineffective "outreach" agency due to the use of this agency by the Ministry administrators to implement other programs. For example, the team was told that village and district extension agents utilized much of their time in regulatory activities enforcing Ministry dictates to farmers regarding acreage restrictions on crops, etc. In addition, these same agents apparently spend some of their time collecting taxes for the government. Another deficiency in the extension program is the extension agents' lack of practical farming experience and their inadequate in-service training in technical subjects and extension methods. These characteristics of the extension program have led the farmers to not trust or respect the extension agents and to be skeptical of the agents' recommendations when they are forthcoming. Also, with the exception of the reoriented extension effort in the Egypt Major Cereals Improvement Project (EMCIP), the Ministry's extension efforts have been further reduced in their effectiveness due to their failure to utilize interdisciplinary technical teams and joint research-extention scientist teams.

C. Egyptian Universities

Similar to the case with the Ministry of Agriculture, at this time the Egyptian Universities do not have any active programs to transfer known BNF technologies to farmers. This inactivity apparently has resulted for many of the same reasons mentioned above for the Ministry of Agriculture. For example, they too have had no saleable "product" or "product package," few members of the Faculties of Agriculture have had practical farming experience and their participation and capabilities in applied field research are limited. Only very recently has a university had any formal channels to governorates, districts, villages and farmers. The Faculty of Agriculture at Alexandria University has just signed a contract with three contiguous governorates for their scientists to participate in outreach activities. Also, the Faculty of Agriculture of Ain Shams University has developed an informal agreement with three contiguous governorates in their area for their scientists to participate in outreach activities.

The team did not learn of any joint Ministry of Agriculture-university outreach efforts and we attribute this primarily to institutional regimentation within both groups. Ministry-

university cooperative efforts in the area of thesis research by university graduate students who had Ministry affiliations is commended. Such programs provide linkage and foster camaraderie.

D. Private Sector

Commercial companies in the private sector play a large and important role in the overall extension effort in some countries. This facet of extension does not exist in Egypt for known BNF technologies. Although it was not definitely determined by the team, this facet of extension may be in effect for other agricultural inputs but would probably be limited to large land holdings such as those under center-pivot sprinkler irrigation, or specialty crops using drip irrigation.

IV. Basic Philosophies

A. General

The following comments describe what we observed to be some of the basic operating philosophies on which extension efforts and related activities in Egypt are based. Each item is followed by a suggested alternate philosophy which, if adopted, should help considerably in developing successful and effective outreach programs in Egypt.

B. The operating philosophy now in effect defines the extension service as a second class agency and considers their scientists to be inferior to the research scientists. These operating philosophies have resulted in the isolation of the extension scientists from the research scientists thereby preventing both types of scientists from making any meaningful contribution to increase farm production and profits via the transfer of known BNF technologies to the farmers.

To have a meaningful impact on increasing farm production and profits via the transfer of known BNF technologies, the Egyptian Ministry of Agriculture and the Egyptian Universities must make some institutional changes. Egypt can no longer afford to base recommended agricultural practices solely on the data of research which is channeled upward to the research administrators who formulate the recommendations which are then channeled laterally as dictates, to the extension administrators and then, again, as dictates, down to the district and village extension agents in whom the farmer has little trust and for whom the farmer has little respect. Adoption of a new and effective operating philosophy of agriculture extension by Egypt is paramount to developing an effective outreach program.

It is recommended that the new operating philosophy incorporate, but, not necessarily be limited to, the following concepts:

1. The extension and research scientists should have equal input and decision-making power in the identification of the farmer's problems as well as in the formulation of recommendations.

2. Implementation of "outreach programs" should be done by interdisciplinary, joint teams of "research and extension" scientists. The teams must be interdisciplinary in order to include scientists with a knowledge of BNF, agronomy, soil science, irrigation practices and weed and pest control. In order to incorporate both the research results and some knowledge of the farmers real constraints on production, the team must include extension scientists and research scientists, either of which should be drawn from the Ministry of Agriculture and from the universities' pools of scientists.

3. Potential members of joint "research and extension" teams must be carefully screened and selected on true dedication to the improvement of agricultural production in Egypt and the improvement of the living standard of the farmers. Careful screening and selection of the scientists on the basis of training in technical disciplines and in extension methods is a necessity. Also at least one scientist on the team should have practical farming experience or considerable experience in applied field research.

4. Extension scientists and research scientists of equal rank should receive equal pay.

5. The team of research and extension scientists should jointly identify farmer problems, conceive and conduct applied field research and demonstration experiments and formulate recommendations.

6. Recommended practices which the teams formulate for farmer use should be placed into practice through salesmanship and not as dictates forced upon the farmers.

7. The research and extension team members should engage only in bonafide research and extension activities. The team members must not be allowed to participate in any government regulatory programs of any kind including tax collection, or in private business as part of their work.

8. Training of research and extension scientists must be an ongoing activity in order to allow the scientists at all

ranks (including district and village agents) to keep current in their disciplines. Training programs must be devised and oriented toward achieving the goal of increasing farm production and profits. The people who formulate training programs should incorporate the above concepts in their programs.

C. The present operating philosophy about "products of science" assumes that any input to agricultural production can be forced into the farmers' management schemes by dictation. This philosophy can lead to very poor results.

The following new operating philosophy is recommended: A "product of science" or a service based on scientific facts must be considered as a marketable item with monetary value. As such, the research-extension team must place the product into the farmer's management scheme by means of their salesmanship, their knowledge of the product and their ability to demonstrate how and when to use it, as well as why it should be used. It is emphasized here that the product of science may very well be a "product package" as described earlier and that the farmer may actually purchase only a portion of the components such as quality seed, quality inoculants, fertilizers, etc. and other components simply may be good technique in seedbed preparation, irrigation water management, etc.

D. The operating philosophy now in effect with regards to the monetary value of and the price which farmers should pay for inputs to crop production possibly has contributed to lack of success with the previous attempt by the Ministry to produce and distribute Rhizobium inoculants to farmers. Government subsidies of several inputs to crop production such as chemical fertilizers is a manifestation of that philosophy. These subsidies can be of real value to farmers and lead to the judicious use of subsidized inputs if the inputs are not over subsidized. However, these subsidy programs can have the effect of turning farmers away from inputs that could potentially benefit their production. For example, the Ministry of Agriculture has a policy of selling Rhizobium inoculants to farmers for L.E. 0.14 for sufficient material to inoculate one feddan. In comparison, the cost of an equivalent amount of inoculum in some countries where the practice is fairly common, ranges between the equivalent of L.E. 0.35 to L.E. 2.50 depending on the particular form of inoculant. The offering of an input, such as Rhizobium inoculant, to Egyptian farmers essentially free of charge could be interpreted by the farmers to mean that the Ministry of Agriculture placed little value on the inoculant. As a result, the farmer may discard it or use it carelessly and improperly.

The following alternate philosophy is suggested: If an input into a farmer's management scheme has the net effect of increasing his production and profits, the input is of real value to him and he is likely to seek it out. Then, it is appropriate to charge the farmer a fair price for that input so long as the price allows for a margin of profit by the farmer. The possibility of a government subsidy is not eliminated but the subsidy can not be unduly large.

E. The present operating philosophy on goals for crop yields suggests that Egypt has assumed that achieving crop yields equal to the yields in other countries, the U.S., for example, is a desirable goal. This philosophy is misleading. For example, some Egyptian administrators told the team that they were satisfied with soybean yields of about 1.0 ton per feddan because it was higher than the average yield in the U.S.A. which is equivalent to about 0.9 ton per feddan. The fallacy in this thinking results from comparing yields of an irrigated agriculture in a warm Mediterranean climate, Egypt, to yields of a rainfed agriculture in a temperate continental climate, the U.S.A., and average yields of full season and second-crop production. The yield potential in Egypt for soybeans is greater than 4.0 tons per feddan which is considerably higher than the present average yield in the U.S.A.

It is recommended that Egypt target yield goals for various crops based on the yield potential of those crops when grown under favorable conditions in Egypt.

V. Recommended Alternate Technology Delivery Systems:

Utilization of all three potential extension (outreach) groups in their appropriate and respective regions and sectors of influence is recommended.

A. Ministry of Agriculture

Institutional changes as mentioned above are necessary prerequisites for the development of an effective outreach program and these changes are highly recommended.

1. The reoriented extension effort in the EMCIP appears to be having some success and it is recommended to serve as the model for the Ministry of Agriculture to use in the development of a BNF extension program. The primary feature is an interdisciplinary, joint research and extension scientist team.

2. Cooperation with qualified scientists from the Egyptian Universities by including them on the teams, is recommended.

B. Egyptian Universities

Likewise, institutional changes as mentioned above will be necessary if the faculties of agriculture in Egyptian Universities are to successfully participate in effective extension of BNF technologies to farmers.

1. It is recommended that the universities utilize the concept of cooperative extension agreements with the governorates in their regions. The primary feature of these outreach programs should be interdisciplinary, joint research-extension scientist teams.

2. It is further recommended that the universities encourage qualified scientists to participate on the Ministry of Agriculture interdisciplinary, joint research and extension scientist teams operating in their regions.

C. The Private Sector

It is recommended that the Egyptian government encourage the private sector to participate in the transfer of known BNF technology.

VI. Implementation

The *Rhizobium*-legume symbiosis is a known technology and soybean is an exotic crop to Egypt, thus, the application of Rhizobium inoculant technology on soybean crops is recommended as the main component of the field production "package" model to be transferred to Egyptian farmers by the reoriented extension programs. Alfalfa, faba bean, berseem clover, peanut, chickpea, etc. can follow as the program develops. When *Azolla*, blue-green algae, associative N_2 fixation, and *Frankia* systems develop into usable technologies they can be implemented.

The following programs are suggested as vehicles for implementation of the extension efforts for the transfer of *Rhizobium* inoculant technology:

A. The Egypt Major Cereals Improvement Project.

B. University cooperative extension agreements with governorates in their regions.

C. Companies in the private sector.

The following steps are suggested for the orderly development of an effective transfer of *Rhizobium* inoculant technology:

A. Develop qualified interdisciplinary, joint research and extension scientist teams.

1. Recruit qualified scientists now in Egypt

2. Train or retrain additional scientists as needed

3. Train district and village agents in BNF technology

B. Import high quality inoculants.

C. Develop storage, handling and distribution facilities for inoculants.

D. Conduct field demonstrations of inoculant technology.

1. Host-plant variety selection

2. Methods of inoculation

3. Forms of inoculants

4. Cultural practice interactions

5. Etc.

E. Report problems to research scientists in order that research be conducted to solve the problems.

F. Formulate a "package program" including the known BNF technology component verified in and adapted to Egypt.

TRAINING

Training and continuous upgrading of skills are essential for any technical program. The Ministry of Agriculture must continue to provide training opportunities. Several avenues of training are available, four of which are discussed below.

Training at the B.S. level should not be ignored. Certainly extension agents and students graduating in agronomy or related areas must have a firm knowledge of BNF and how it is applied at the farmer level. The training of B.S. students is the responsibility of the universities and the new programs to include on-farm experience are to be commended.

The training of current extension agents will partially be handled by the Egypt Major Cereals Improvement Project and should include BNF technology.

Specific BNF technology training should include:

1. Importance of BNF at the B.S. and extension agent level
   - increasing livestock forage and protein for human consumption
   - conserving nitrogen fertilizer
2. Factors limiting BNF
   - drainage, irrigation, fertility, temperature, economics
3. Storage and handling of inoculants
   - precautions needed, facilities needed, distribution, timeliness
4. Inoculation of legume crops
   - seed, soil and other methods
   - forms of inoculants
   - time of inoculation
5. Preparation of field for planting
   - preirrigation, seedbed preparation, pesticide and fertilizer placement

6. Checking for field nodulation

   - how to sample a legume for nodule evaluation

   - identification of well-nodulated legumes and knowledge concerning the differences in various legume nodule morphology, and distribution patterns

   - recognition of effective (red or pink), ineffective (white) and old (green) nodules

7. Demonstration plots

   - how to perform simple and effective BNF field demonstrations at the farmer level.

Well-educated and highly capable people were visited during this evaluation, yet many problems can be corrected with improved and additional training. Some of the opportunities for both current and improved training programs focus on the following challenges:

1. Can known and available technology be applied in both field and laboratory conditions?

2. Can cleanliness and maintenance be improved?

3. Can field research be better organized and executed?

4. Can cooperation between scientists of disciplines pertinent to BNF, between the universities and the Agricultural Research Center, and between all researchers and extension be improved?

5. Can expertise in the production, storage, distribution, merchandizing, and testing of quality inoculants be developed and maintained?

Scientific Exchange

The exchange of scientists between institutions to include both out-country and in-country exchange should be expanded.

Adequate training in agronomic field research is rare among microbiologists. Adequate training in BNF technology is rare among agronomists and soil scientists. Travel by Egyptian scientists to the U.S. may provide training in the following areas:

1. Technological areas for which inadequate opportunity exists in Egypt.

2. Implementation of current technology in the field and at the farm level.

3. Participation in cooperative research and the organization of research.

4. Improving research application and extension to solve BNF problems in Egypt, especially through the transfer of technology from the research lab through the extension service and to the farmer.

Travel by U.S. scientists to Egypt should be part of the scientific exchange for similar reasons.

In-country scientific exchange to:

1. Organize a national BNF program.

2. Transfer technology between institutions.

3. Develop cooperation between institutions.

4. Identify areas where concentrated research effort is needed.

5. Organize uniform field trials of BNF technology.

6. Compare results in similar experiments.

7. Change emphasis on BNF research when appropriate.

8. Insure the knowledge is transferred to extension and is implemented at the farm level.

Post Doctoral

Post Doctoral training of Egyptian educated Ph.D.s is essential. A well-managed and clean microbiology laboratory with an organized research effort could be encouraged by proper budgetary support and post doctoral training opportunities. Additional training and a chance to work with a research team may help develop BNF laboratories equal to the best in the world. Further training should include:

- field plot technique and maintenance

- equipment maintenance

- housekeeping and cleanliness

- organization of a research program including cooperating with other disciplines

Additionally the student should be well-trained in the traditional post doctoral pursuits.

Post Graduate

Post graduate training in the U.S. and other countries in an active BNF laboratory can be valuable for the same reasons as listed above.

Graduate training in both the theoretical and applied aspects of BNF is required. However, the main research emphasis should include the more applied aspects of BNF and include field problems. This does not imply that basic or theoretical studies should not be encouraged, but indicates the direction in which most of the students should be pointed. An approximate figure of 75 percent applied thesis problems to 25 percent basic is suggested.

Students in the more basic research areas must interact with those in the more applied areas and include some course work in agronomically related fields to gain some understanding of practical problems. Students in the more applied research areas must take some basic science courses as well as those in the areas of crop, soil and water sciences, field plot technique, and statistics. Field research must directly involve the student from the experimental design and implementation stages through data summarization, including active student participation in all phases of machinery and plot maintenance.

Specialized Training

Specialized training may include several different types of training not previously mentioned. The development of expertise in all aspects of legume inoculant production is much-needed and must be given top priority. Egyptian scientists directly involved with inoculant production must have the opportunity to train in well-run foreign legume inoculant production facilities. The philosophies and practices of any inoculant production facility in Egypt must achieve correct cleanliness, proper carrier handling, supply ordering, media preparation, culture collection handling, sterilization, mixing, packaging, storage, distribution, marketing, and how these various components are systematically compartmentalized in a modern factory. Several commercial inoculant producers in the U.S. would be willing to cooperate if appropriate arrangements can be made.

Other specialized training may include training sessions held on BNF technology similar to the *Rhizobium* training sessions organized by NifTAL. Beltsville and NifTAL projects can assist with some training needs. More intensive training sessions may be necessary for specific subject matter extension specialists.

RESEARCH

The research programs in biological nitrogen fixation in Egypt appear to be carried out by well-trained scientists with careful attention to detailed observations and measurements. Sometimes vital information is missing; the problems researched appear to be more related to the current scientific literature than to problems observed in the field. The techniques used are standard, but sometimes opportunities to adapt them in innovative ways to solve problems are overlooked. Much of the research adds to the data available on microbiology and biological nitrogen fixation, but the usefulness of such data to Egyptian agriculture is difficult to assess or put into practice. The research activities appear to be fragmented because each investigator follows a special interest, or because funding support is sporadic, and inadequate. Cooperation and coordination is lacking. Biological nitrogen fixation research in Egypt can be categorized into five (5) systems; (1) Rhizobium-legume, (2) (2) Azolla, (3) Associative N_2-fixing bacteria, (4) Blue-green algae, and (5) Frankia. Field research is needed to test the BNF technology developed in the laboratory and greenhouse. Some basic research conducted in the laboratory and greenhouse is needed to develop scientific understanding and to provide the basic information for improving BNF technology. Some basic research (for example, presence and survival of Rhizobial strains in different soils) needs to be done in the field. Most research should be related to problems that have been identified in the field, and the methodology should be no more sophisticated than needed to solve the problem.

The team feels that currently the total scientific effort in BNF research should be apportioned as shown on the chart following on page 31.

A. Rhizobium-legume system

This system is well-known and the technology for applied field use has been developed and is functioning in other countries, but has not been adapted and transferred adequately in Egypt. A current summary of the Rhizobium-legume technology is presented by Dr. John Halliday, University of Hawaii in Appendix 1.

N_2 fixing System	Total % of BNF Effort	Research Activity (%) within each system: Field	Laboratory and Greenhouse
1. *Rhizobium*-legume (grains, forages)	60 to 80	60 to 80	20 to 40
2. Azolla (flooded soils only)	10 to 20	40 to 60	40 to 60
3. Associative N_2 fixing bacteria (cereals, cotton, other)	5 to 10	20 to 40	60 to 80
4. Blue-green algae (flooded soils only)	3 to 10	30 to 50	50 to 70
5. *Frankia*-non legume and others (*Casuarina*, other)	2 to 5	10 to 30	70 to 90

Numerous field trials should be initiated to help develop the sound field research data concerning inoculation of various legume crops in different Egyptian soils on which to base recommended inoculation practices. Also, research is essential before the *Rhizobium* inoculant production system for Egypt can be formulated. Decisions based on research need to be made concerning successful field inoculating methods, appropriate inoculant carrier, and sterile or non-sterile carrier. Certain recommended research projects considered to be of major importance will be outlined below but are not intended to be all-inclusive.

1. Need-to-inoculate trials: Determine, on an individual basis, the need to inoculate for each legume beginning with recently introduced legumes, but including all pulse and forage legumes. Field inoculation trials should be conducted to determine the response to superior *Rhizobium* strains for each crop (see BNF Activities in the EMCIP section of this report).

2. Inoculant methodology trials: Develop field methods that reliably nodulate field legumes where soil-borne *Rhizobium* numbers are not satisfactory (see BNF Activities in the EMCIP section of this report). It is important for this to be determined before an inoculant production capability is initiated in Egypt.

3. Inoculant carrier evaluation: Research needs to be continued on the evaluation of possible carriers to be used for inoculant production in Egypt. Several local sources as carriers have been investigated including a mixture of soil and ground berseem straw. Preliminary research has been initiated but a uniform evaluation of all sources, also using a known effective peat carrier, should be conducted and must include measurement of nodulation establishment using marked strains in field trials.

   When local inoculant production is initiated it is very important to be assured that the quality of such inoculant be consistent. The choice of an inoculant carrier is thus an important consideration to be determined before initiating inoculum production. If an imported carrier is determined to be superior it would be advisable to import the carrier and produce a quality inoculant in Egypt with locally produced cultures.

4. Carrier sterility: It is advisable to not only evaluate the various carriers but also compare the performance and cost of the carriers in a sterile vs. non-sterile form.

5. Field testing of Rhizobium strains: Collect and maintain local and foreign strains of rhizobia for the legumes produced in Egypt and evaluate these strains for nodulating ability with various hosts, compatibility with cultivars, and the field environment, nitrogen fixation and effect on yield.

6. Ecological studies on rhizobia in soil: Evaluate persistence, effect on other legumes in the cropping systems, tolerance to various soil stress conditions (high pH, salinity, low phosphorus, coarse textured soils, flooding, low organic matter, etc.).

B. Azolla-flooded crop system

Azolla is a small water fern that contains a blue-green alga intimately associated with the fern. The blue-green alga fixes N_2 resulting in the combined plant being high in protein. Azolla has been divided into six species--some of which are cold-tolerant but the growth rate increases as the temperature increases up to about 30 to 35° C. Azolla has been used as protein feed for ducks, chickens, pigs and other animals, but the intake usually is restricted to not more than 20% of the diet. Azolla has also been used as a green manure to provide nitrogen under flooded conditions, for rice and taro. Each crop of Azolla can provide organic matter and

about 30 Kg N/ha to the crop when worked into the soil. Present methods of using Azolla are very labor intensive and require careful management. The Azolla is grown in nurseries and 1 to 5 T/ha (fresh weight) are added to the shallow water on the field. After growth has covered the area (usually about 20 to 30 days), the Azolla is worked into the soil. If the rice crop does not shade the water, another Azolla crop may develop. Good management requires protection from wind which blows Azolla against the bunds, protection from insects and fish, an adequate supply of P and careful water management.

Research needed includes the following:

1. Methods of culturing Azolla in nurseries and managing Azolla in the fields.

2. Selection of productive species or strains for Egyptian conditions (southern Egypt may have different requirements from northern Egypt, also Southeast Asian strains may not be adapted to Egypt.

3. Determination of contribution to cropping system (N supplied to crops, weed control, competition, etc.)

C. Associative or Non-symbiotic N_2-fixing bacterial systems.

Many N_2-fixing bacteria exist in Egyptian soils. Azobacter is plentiful in Egyptian soils, with recent studies indicating that many other bacteria in the root zone of grasses have been found that fix N_2 only under low oxygen tension. These include Azospirillum, Klebsiella and some bacilli.

All N_2-fixing systems require energy, but the associative bacteria must compete with other bacteria for their energy and are not protected by a root nodule like Rhizobium and Frankia. Because these bacteria require energy for growth and N_2-fixation (they fix N_2 to supply only their own needs), only about one unit of N is fixed for each 100 units of sugars used. Therefore, to fix 10 Kg N the plant or soil must provide at least 1000 Kg organic matter against the competition of all the other soil microbes, and the system must have no available combined nitrogen.

Research needs on the associative nitrogen fixing bacteria include the following:

1. Repetition of field trials of Azospirillum inoculation of wheat and maize to determine the consistency of yield increases and the relation to N fertilizer response.

2. Trials on other cereal grains and possibly other non-legume crops.

3. Effective methods of inoculation and the need to reinoculate.

4. Determine the ability of N_2-fixing bacteria to colonize roots. Determine whether differences exist between cereal species and cultivars.

5. Land requirements for nurseries.

D. Blue-Green Algae system

Considerable work has been done with this system using rice grown on flooded soils. Favorable results have been reported that indicate the use of blue-green algae can reduce the need for nitrogen by one-half. This system is also used to some extent in India, but the system does not appear to be widely used by farmers.

Research needs include:

1. Confirmation of yield increases by agronomists trained to use the blue-green algae technology and with appropriate fertilizer nitrogen controls.

2. Investigations to determine why farmers are reluctant to adopt the blue-green algae technology.

3. Nitrogen balance studies on greenhouse and field systems to determine the contribution of each component of the nitrogen supply (and losses).

4. Basic studies on strain selection, nutrition, culturing, pest protection and harvesting of blue-green algae.

5. Land requirements for nurseries.

E. Frankia-root nodulated non-legume system.

At present, only perennial, woody plants are known to have this N_2 fixing system. The most common genus in Egypt with this system is Casuarina, although some Alnues may be found. Little is known of the value of these plants in farming systems, but obvious uses include providing windbreaks, firewood and building material.

Research needs include:

1. Surveys to determine plants that have this system with observations to indicate their potential value in farming systems.

2. Studies and selection of both host plant and endophyte to improve and broaden the utility of the plants and to select types that fit different needs.

3. Studies to determine ways to culture the endophyte and to produce inoculants for tree nurseries.

All of the systems have intriguing research problems. The greatest and most rapid increase in total agricultural production is likely to result from maximizing the use of the Rhizobium-legume system. The greatest potential benefit would derive from a nitrogen-fixing system that could provide the major portion of the N needs of cereals. The Azolla and blue-green algae systems provide N only for flooded crops and the cost in labor, management, space (land) and time is considerable The associative bacterial system has not been domesticated so it is reliable and consistent.

Agricultural scientists in the U.S.A. and their institutions are interested and willing to work cooperatively with Egyptian scientists on many of these research areas.

RHIZOBIUM INOCULANT

The current inoculant production facilities at both the Ministry of Agriculture, Giza and Sakha were visited by the BNF team. The same conditions in the laboratories as observed and reported by Dr. Rodney J. Roughley (Microbiology Consultant) in his visit, April-May 1976 (Improvement of Field Crops Productivity - Egypt: The large-scale production of Rhizobium inoculants for soybean, AG:DP/EGY/71/591, Working Paper 12) still exist. No apparent effort had been made to implement the recommendations of Dr. Roughley.

The inoculant production system has been changed to utilize two Jiffy peat discs in an autoclavable plastic cup. Two discs are sterilized in the cup before the Rhizobium culture is added to the cup. The Jiffy peat discs adsorb the culture to provide the material which is to be used as a seed-applied inoculant. The user must remove a plastic net from around each disc, crumble the inoculant into as small a particle as possible and attempt to adhere this inoculum to the legume seed.

The assessment of the team, and the Egyptian Ministry of Agriculture is that this inoculant is not satisfactory and production in this form should be discontinued. This assessment is supported by the lack of sufficient nodulation in field experiments.

Extensive contamination and insufficient Rhizobium survival in the acid peat discs, in addition to the absence of a quality control program were reported to be the main reasons for failure. Extensive nodulation has been achieved in soybean field inoculation trials sponsored by INTSOY (International Soybean Program). These inoculation trials, ISRIE (International Soybean Rhizobium Inoculation Evaluation) have been successful at the Ministry of Agriculture - Giza and Nubaria, and the University of Monoufeia (Table 1). ISRIE trials and other inoculation trials have utilized the granular soil inoculant which provides approximately 2.3 Kg/Fd inoculant in contrast to 93 g/Fd supplied with seed applied inoculants.

A. Recommended Rhizobium Inoculant Program

Based on these conditions and results, the following BNF Program concerning Rhizobium inoculants is recommended.

1. Import quality legume inoculants - It is essential that high quality inoculants capable of producing satisfactory nodulation on soybeans be imported immediately. With rates of nitrogen fertilizer currently used on soybeans, from 60 to 90 Kg/Fd, a large economic savings for the Egyptian

Table 1. Soybean yield with ISRIE inoculant trials in Egypt using granular soil inoculant.

Location	Yield (Kg/ha)			
Location	Control	Inoculant[a]	Inoculant and Nitrogen fertilizer[b]	Nitrogen fertilizer[c]
Nubaria	513	2182	1356	475
University of Moncufeia	5209	6543	5001	5126
Giza	850	3619	3344	1141

a - Nitragin Soil Implant, 5 lb./acre

b - Nitragin Soil Implant, 5 lb./acre plus 25 lb. N-fertilizer/acre at planting.

c - 25 lb. N-fertilizer/acre at planting plus 75 lb. N-fertilizer 5 weeks after planting.

government would be realized by utilizing a soil inoculant in place of the nitrogen fertilizer, even if no yield advantage was achieved. The use of inoculants on other legumes would also be required when plantings are on new land for that particular legume. Nitrogen fertilizer is currently being used with other commonly grown legumes such as faba beans and berseem clover. The potential for both economic savings and yield advantages by changing the farming practice from nitrogen fertilizers to inoculant use should be investigated. Importation of high quality legume inoculants can meet the immediate need for inoculants and also help develop the market demand. If desired, an Egyptian "private" label might be available on this imported legume inoculant.

2. Research to be initiated concerning inoculant production, distribution, and methods of inoculating - Research activities concerning these problems have been listed in the Research section and the EMCIP section of this report. It is essential that these answers be obtained so an appropriate inoculant production capability can be designed.

3. Develop inoculant production capability in Egypt - As the market demand develops through use of imported quality inoculants, and research has provided answers to allow the production of a reliable inoculant in Egypt that will perform in Egyptian fields, an improved inoculant production capability should be developed.

4. Evaluate the potential for increasing legume yields and minimizing the nitrogen fertilizer needs for all legumes in Egypt - A field survey should be conducted to investigate the amount of nodulation and effectiveness of nitrogen fixation for all legumes presently grown in Egypt. This would include sampling in farmers' fields for nodule abundance, isolation and nitrogen fixation testing of these indigenous strains. Field trials with known effective strains to evaluate yield response in comparison to sufficient nitrogen fertilizer should be conducted for each legume crop.

B. Inoculant Production Alternatives in Egypt.

1. Legume inoculant producer - Local production of legume inoculants in Egypt should be economical. Egyptian production would eliminate the concern for inoculant quality deterioration during the shipping and custom entry period that imported inoculants must withstand.

Inoculant production would best be served by a joint venture between the Egyptian Government (or company within the government) and a foreign private company with expertise

and proven experience in the production of quality inoculants. This would provide the system by which quality inoculants could be produced in Egypt within the shortest time possible. Experienced management must be assured to expedite the problems involved with inoculant production, marketing and sales.

An alternate approach would be for an Egyptian private company to develop the inoculant production capability either independently or in cooperation with an existing foreign inoculant producer. Extensive training would be required in such a venture. Also, close cooperation between the Ministry of Agriculture and the private industry would be required. Thus the joint venture is the preferred approach for rapid development of quality Egyptian inoculant production capability.

The location of the inoculant production facility in the government joint venture proposal would have to be determined by the cooperating parties. The present inoculant production facility at Sakha would require extensive internal structure redesign to become an efficient inoculant production facility.

2. Production of legume inoculants - When an inoculant production plant is built the following recommendations should assist in the planning and implementation stages.

The present inoculum needs appear to be for approximately 100,000 Fd of soybeans, all legumes being planted in the new land development areas plus unknown needs for legumes currently grown on old lands. The following inoculum production facility should be designed to meet the current needs plus allow for expansion as required.

a. Culture production

(1) Rhizobia culture collection
Strains of tested effective rhizobia for each of the legumes grown in Egypt must be maintained in a satisfactory culture collection. Cultures of rhizobia to be used as mother cultures are best maintained freeze dried in glass ampoules. From these a set of working cultures should be prepared and maintained on agar slopes of yeast extract mannitol agar (YMA). This system reduces the frequency of subculturing and the likelihood of undesirable mutations.

Strains in the collection should be both proven effective strains from other culture collections

and also isolated Egyptian strains from various areas of the country. These strains should be tested for nitrogen fixation and performance in both greenhouse and field trials evaluated with the best strains approved for culture production.

(2) Starter cultures
Using the approved strain(s), starter cultures are prepared from the mother culture agar tubes and may be streaked on agar slopes within a 500 ml side arm Roux flask. After appropriate growth is obtained on the agar flask, sterile water is aseptically transferred into the flask via the side arm and the growth is gently removed by swirling the flask taking care not to wet the plugs.

To inoculate the fermentation vessel, the connecting rubber tubing is cut aseptically and the flask connected directly to the surface sterilized metal inoculation valve. The valve is sterilized by direct flaming and the tube connected while the valve is still hot. The starter is added to the fermentation vessel when the valve has cooled to room temperature. The concentration of starter may vary from 1 to 5%. Shorter incubation time will be achieved with larger inoculum volumes.

Multistrain inoculants for each particular legume host may be produced by growing single strain cultures and mixing the final broth of each strain immediately before incorporation into the inoculant carrier.

3. Fermentation vessels - Large-scale fermentation equipment is not required to produce sufficient culture for initial projected production needs. A culture production system utilizing fermentation vessels of 10 to 50 liter capacity can provide good serviceability when designed with simple hand operated controls. Automatic fermenters are more difficult to maintain in continual operation. Without reliable manufacturer's service rapidly available, breakdowns result in time-consuming trouble shooting and production down time waiting for replacement parts. Automatic fermenters are not recommended.

Fermenters fabricated from steel drums fitted with brass inoculating, air and sampling inlet and outlet parts are described by Date, R.A. and R. J. Roughley, Preparation of Legume Seed Inoculants, IN: A treatise on Dinitrogen Fixation. Section IV: Agronomy and Ecology. Hardy and Gibson (eds.), John Wiley and Sons, N.Y. This system

utilizes cotton wool packed metal cylinder filters to sterilize the air from a compressed air supply. The vessel with air filter attached and two-thirds filled with liquid medium must be sterilized before inoculated with the starter culture.

Rhizobia strains grow well at 28° C. As the fermenters are not temperature controlled they should be incubated in a temperature controlled room. If the ambient temperature is below 28° C, thermostatically controlled heaters may be used to maintain adequate temperature.

4. Mixing of culture with carrier.- The choice of type of carrier and method of carrier preparation must be determined as indicated in the earlier section concerning rhizobia research recommendations. This also includes the choice of sterile or non-sterile carrier. The method of broth incorporation will depend upon the type of carrier but most importantly upon the choice of sterile or non-sterile carrier.

   a. Non-sterilized carrier

      Broth is added to the processed carrier in the appropriate quantity as determined by previous experimentation. The carrier should be agitated in a mechanical mixer while the broth is being slowly added. Mixing should be conducted in a batch process with appropriate quantities of carrier, $CaCO_3$ (if the pH of the carrier needs to be increased to 6.8-7.0), and broth culture. The carrier and culture are blended thoroughly until all broth is absorbed and evenly incorporated. If $CaCO_3$ is utilized, the quantity of $CaCO_3$ necessary to raise the pH of one batch of carrier, is added to the required quantity of broth and agitated to dissolve as much $CaCO_3$ as possible. Mixing is continued to maintain a suspension during the time broth is added to the carrier.

   b. Sterile carrier

      Each inoculant package containing the sterile carrier may be inoculated with a measured amount of broth using a hollow needle connected to an automatic syringe which in turn is directly coupled to the fermenter. The contents should be shaken or manipulated to assure uniform culture distribution within the carrier.

5. Curing the mixed inoculant - Dried, non-sterile peat will release heat due to a chemical heat-of-wetting when the culture is added in the inoculant mixing stage. The mixed inoculant should be dispensed into metal trays with 10 cm sides and cured at 26° C for 4 - 7 days for heat to escape. This is a curing phase and all efforts to minimize drying should be undertaken. The surface should be covered with polyethylene film to reduce moisture loss from the surface. Maturation in trays also reduces growth of molds after packaging.

6. Packaging - Rhizobia have a small but definite requirement for air during storage. Reports which suggest satisfactory survival in sealed containers probably utilized containers which had a large air space. In sealed containers, cell numbers decline during the first week. Typically the mean weekly logarithmic decline in cell numbers with free access to air is 0.05 and in sealed cans 1.2.

   The best packaging material for inoculants is thin polyethylene bags. In non-sterile systems pin-holes are added to the bag to ensure adequate aeration. Under these conditions the rhizobial population should increase at least 10-fold within the month after mixing.

Quality Assurance

An effective quality assurance program for all inoculants used in Egypt (either produced within Egypt or imported) is very important to a successful inoculant program. It is recommended that the function of quality evaluation be conducted by the soil microbiology section of the Ministry of Agriculture. The necessary microbiology laboratory and plant growth facilities should be available in the EMCIP microbiology laboratory to be built at the Ministry of Agriculture - Giza, but space in another location will be needed for the activities of an Egyptian Inoculant Research and Control Service.

1. Broth culturing - Quality assurance must begin with the selection and maintenance of mother cultures, and include control of the broth before addition to the carrier and the finished product. All final broth batches must be given a lot number. This lot number must be conveyed to each batch of inoculant and the packages of inoculant prepared from that lot.

   Broth cultures should provide at least 1 x 10^{9} rhizobia/ml. The time required to achieve this count will depend upon the growth medium, amount of inoculum and whether the strains are fast or slow growing rhizobia. The broth should be examined microscopically to assure that the cells are typical, the broth is free of contaminants and the total

viable count meets the minimum standard of 1 x 10^9 cells/ml. Serological tests will verify that the correct strain is present before mixing.

2. Cured inoculants - Quality in legume inoculants depends upon three factors: (a) the ability of the Rhizobium in the inoculant to nodulate the host plant, (b) the ability of the strains to fix nitrogen efficiently, (c) the presence of an appropriate number of rhyzobia to successfully nodulate the legume in the field. One can determine the number of viable rhizobia in the laboratory, however, the only way to determine nodulation ability and N_2 fixation efficiency is to inoculate seed of the specific legume and grow it in a nitrogen-poor substrate under favorable conditions. The final evaluation of an inoculant or method of inoculation is field testing under common farming practices and local stress conditions.

With batch mixing of non-sterile carriers the cured inoculant should be tested for proper moisture content, as determined by previous experimentation. This is required because of the fluctuation in initial moisture content of the dry carrier.

The number of rhizobia in an inoculant can be determined by suspending an aliquot of the sample in sterile water and making appropriate dilutions and plate counts. The colonies of bacteria can be counted after incubation at 28 to 30° C for 4 to 5 days in the case of fast growers such as R. phaseoli or 7 to 8 days with slow growers such as R. japonicum, R. lupini, or cowpea rhizobia. This plating method for viable colony forming particles is most suitable for sterile inoculants but can also be used with good quality non-sterile inoculants with the aid of contaminant restrictive compounds such as Rose bengal and oligomycin. Cultures in unsterilized carriers should contain a minimum of 1 x 10^8 rhizobia/g carrier and in sterilized carriers the minimum number should be 1 x 10^9 Rhizobium/g.

Viable counts of rhizobia can be effectively evaluated with the Most-Probable-Number technique (MPN). Appropriate dilutions of the inoculant may be used to inoculate plants grown in test tubes, bottles, or growth pouches. The number of tubes which bear nodulated plants after a 4 or 5-week growth period can then be used to statistically estimate the number of rhizobia present. Clean plant growth facilities are essential for these inoculant quality control testing procedures.

D. Inoculant Storage and Distribution

Inoculum is a perishable product and care must be exercised in maintaining the best storage and handling conditions.

Inoculant quantity is best maintained under refrigeration. A cold room at the inoculant storage facility is essential. Egyptian temperatures do not decline sufficiently to allow the ambient temperatures to meet these temperature needs, as occurs in the cool, temperate regions of the world.

Care should be exercised in expediting inoculant shipments to regional distribution points and to the farmer. A system by which the farmer must come to the inoculant production site to obtain his inoculant supply is not feasible in an improved BNF program designed to reach the Egyptian farmers. A network of regional and local inoculant distribution points must be established to meet these needs. This is a major constraint in the rapid development of an improved inoculant program in Egypt.

Storage of inoculant at these regional and local centers should be for as short a time as possible, but still inoculant inventory must be available to the farmer at the time he is ready to plant. Inoculant storage systems at these centers should be out of the sunlight and in as cool a place as possible. Research in inoculant storage containers, storage locations and an evaluation of the Egyptian farming system is recommended. Possibly inoculant storage in underground earthen structures such as those used by farmers for seed storage may be adequate. Cooler temperatures are obtained in the soil and research may develop such an underground system that would be effective, especially at the village and farm level.

Expiration dates should be placed on the inoculant package and must be strictly enforced. Expiration dates should be used to prevent carry-over of an inoculant to a following year's planting season. In general, inoculants should be used when as fresh as possible. Inoculant production should be scheduled to allow sufficient production and distribution before the respective legume planting period with minimum storage times.

BNF ACTIVITIES IN EMCIP

Background for BNF activities in EMCIP

Most farmers in Egypt do not know that legumes fix nitrogen in their root nodules. Yet traditional farming systems almost invariably include legumes, as do many modern farming systems. Legume cultivation results from recognition by farmers that legumes are valuable components in farming systems rather than from intentional exploitation of BNF.

BNF technology based on legumes has two major aspects: (1) "the use of legumes" related to the deliberate inclusion of legumes in cropping systems to derive benefits from BNF and the crop produced; (2) "Inoculation technology" relates to the intentional use of specific practices to optimize nitrogen fixation by the legumes. This distinction emphasizes that currently legumes are used widely with less than maximal benefits from BNF because of deficient symbiotic associations and soil factors which limit N_2 fixation. Productivity could be increased by using appropriate technology to assure effective symbiotic N_2 fixation by legumes in farming systems currently in use. Even greater gains in productivity and reduced fertilizer nitrogen requirements can be realized by introducing legumes in innovative farming systems, e.g., soybeans in agronomic systems or fast-growing leguminous trees in agroforestry systems. Production gains will be greatest if the use of legumes is always complemented by appropriate inoculant technology. Legumes can only benefit fully from BNF when the cultivar is an efficient N_2 fixer and is supplied with efficient Rhizobium that are genetically compatible, allowing root infection and effective nodulation to occur promptly and persistently. Effective nodulation functions by providing nitrogen to meet the needs of the legume throughout the production period of the crop.

Direct benefits from the use of legumes in farming systems arise from the economic value of the crop for food, feed, forage or fuel. Indirect benefits accrue from (1) reduction in the risk of crop failure due to disease, insects, weather, or price (2) contribution of nitrogen to succeeding crop and (3) interruption of pest buildup in non-legume crops. Inoculant technology involves the following:

I. Selection and use of strains of Rhizobium that are (a) compatible and effective N-fixers with the desired legume cultivars- (b) able to survive and multiply in the root zone in the soil, and (c) able to form nodules that provide nitrogen to the plant in the presence of ineffective strains that

nodulate the cultivar; (d) ability to multiply in liquid culture to high population densities, (e) ability to grow to high population density in the carrier (usually peat), (f) ability to survive at high population during packaging and distribution, (g) ability to survive during inoculation by soil or seed application, (h) ability to multiply and nodulate early and remain effective throughout the production period.

II. Inoculant Production

This subject is covered in another section and should not be a responsibility of the EMCIP laboratory, but the EMCIP laboratory should be responsible for obtaining high quality inoculant for all experiments and field trials and participate in inoculant quality assurance activities.

III. Inoculation Methodology

The prime objective of inoculation of legume seed with *Rhizobium* inoculants is effective nodulation of the legume host plant. To accomplish this objective a reliable procedure must be developed for each crop. This procedure involves the following:

A. Use of high-quality inoculant. High quality inoculant provides large numbers of effective, competitive *Rhizobium* per gram of inoculant.

B. Proper application of inoculant. (1) Large numbers of *Rhizobium* per seed provide assurance against nodulation failure due to stress conditions. (2) Reliable and convenient as possible for the farmer, whether by applying directly to the soil (suspension or granular) or by coating the seed (pelleting or slurry). Application rates should be cost-effective, but not simply based on lowest cost. (3) Other agronomic practices must be appropriate; tillage, irrigation, drainage, fertilizer, pest control, etc.

Specific Suggestions for the EMCIP Microbiology Program

1. Conduct need-to-inoculate trials for all legumes of interest to EMCIP, using the NifTAL (University of Hawaii) protocol and making use of the marked strains, when appropriate. The protocol for the International Legume Inoculant Trials (INLIT) is available upon request from Dr. John Halliday, NifTAL Project - University of Hawaii, P.O. Box "O", Paia, Hawaii, U.S.A. A copy will also be sent to Dr. Dessouki

and Dr. Everson, Co-Project Directors, Preliminary trials to determine the presence of soil rhizobia capable of nodulating introduced legumes can be made on carefully conducted pot trials. Such trials require clean techniques and great care to avoid contamination.

2. Conduct inoculation methodology trials for grain and forage legumes. Begin with introduced legumes where appropriate Rhizobium are absent or present in very low numbers in the soil, and expand to all other legumes as time permits. The objective is reliable, effective nodulation that provides the nitrogen requirements of the legume with application techniques as convenient as possible for the farmer. Application techniques for hand planting may need to differ from those used for machine planting.

   Obtain high quality inoculants from two or three sources from which commercial quantities are available. Conduct trials with the following treatments (not necessarily in one experiment):

   (a) Inoculation rates - X (recommended application rate), 4X, 12X.

   (b) Irrigation schedules.

      (1) Pre-planting, post-planting farmer practice, post-planting two times the farmer practice.

      (2) Normal farmer schedule, increased frequency, decreased frequency.

   (c) Application methods - slurry treatment of soaked seed, slurry treatment of dry seed, granular in row or hill, suspension in row or hill, suspension in irrigation water, other.

3. Work with Extension and Training to explain how to handle and use perishable inoculants. Consult with extension agriculturists about any failures in the field and conduct research as needed to solve these problems. Prepare how-to-do-it guides and extension programs, on legume inoculation.

4. Evaluation of inoculant quality for Egyptian conditions.

   (a) Assure quality by appropriate tests - MPN plant dilution counts of rhizobia, plate counts, or serological typing (if feasible).

(b) Field evaluation.

5. Select for improved strains of Rhizobium when a legume or legume cultivar shows consistent yield increases when fertilizer N is added to well-nodulated plants, or if consistent nodulation failures are found.

6. Identify and solve nodulation problems that may occur when several legumes are used in a cropping system on the same field. For example, in Australia and California, subterranean clover nodulated well, but was completely ineffective (no N_2 fixation) due to white clover Rhizobium present. Pelleted inoculant and more competitive effective strains for subterranean clover provided good effective nodulation. There may be similar problems with berseem clover and alfalfa; they should be investigated.

7. Conduct agronomic trials of cereals inoculation with Azospirillum or other N_2 fixing bacteria to ascertain whether significant yield increases can be obtained.

8. Insure the competitive grants program of EMCIP includes BNF research.

Equipment and Design for EMCIP Soil Microbiology Laboratory

The basic floor plan design and amount of space for the soil microbiology laboratory is adequate. However, slight modifications are suggested.

The door of the laboratory should open into the outer office as well as the hall.

To minimize contamination and promote use, one of the "offices" should be used for a plant growth room for plant testing of Rhizobium strains, inoculant testing, and other plant growth tests which cannot be performed in an open greenhouse setting. This room should be fitted with incandescent and fluorescent lights, benches for support of pots and Most-Probable-Number Rhizobium tests, and a temperature and light control system. If growth chambers are used in lieu of the plant growth room, they should be placed in the same "office" space. Any additional growth chambers needed might be placed in the area adjacent to the greenhouse. However, growth chambers would be more difficult to maintain and repair in Egypt and are not suggested.

The laboratory benches must have tops of stainless steel or synthetic slate, and include storage drawers and cabinets. The floor covering must be smooth and non-porous. Seamless vinyl is preferred, but vinyl or asphalt tiles are satisfactory.

A separate room would not be required for culture work if the laboratory is kept clean and laminar air flow hoods are used. Instead, an area of the lab can be enclosed with partitions and/or cabinets (at least 2 meters high) to isolate critical work from routine work.

Basic equipment needs for the microbiology laboratory are listed below.

1. Self-generating steam autoclave. Inside dimensions of at least 60 cm in diameter and 120 cm long.

2. Two refrigerators, with freezing compartments, 1 for routine laboratory use and 1 only used for culture storage.

3. Laminar air flow hood of stainless steel with a design to include prefilters and lights.

4. Semi-micro Kjeldahl nitrogen apparatus to include a large supply of parts and glassware.

5. Chemical fume hood.

6. Three balances; 1 - 2 Kg $\pm$0.1 g, 1 - 200 g $\pm$ 0.01 g, 1 - 20 g $\pm$ 0.0001 (analytical).

7. Balance table for the analytical balance.

8. Two microscopes; 1 stereoscopic with above and below lighting with 4 variable powers or zoom lens up to 100 X, and 1 compound microscope to include bright field and phase contrast with 4 objectives up to 1000 X.

9. 2 Shakers, rotary type, 1 large and 1 small.

10. pH meter.

11. Microbiological incubator, 60 cm wx 80 cm dx 100 cm h

12. Water bath for hot agar. 25 cm x 50 cm

13. Small centrifuge.

14. Pipette washer.

15. Laboratory benches and cabinets as roughly outlined in the following.

16. Assorted laboratory glassware to include petri dishes, pipettes, etc.

17. 4 laboratory carts, laboratory stools.

18. Water distillation unit.

19. Small spectrometer, similar to a Bausch and Lomb Spec. 20.

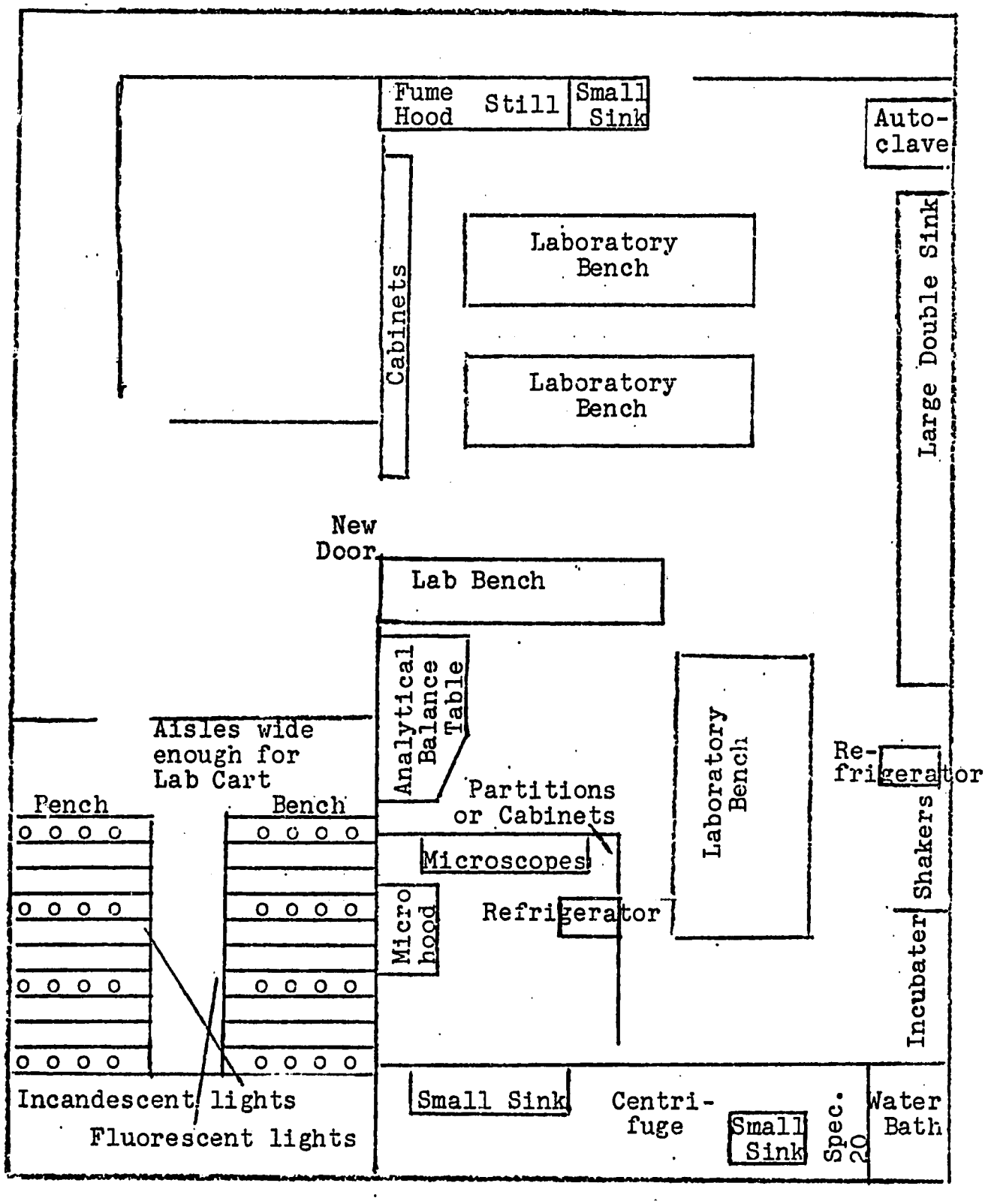
20. Incandescent and fluorescent lights, benches for pot support timing mechanism for lights, and air conditioner for plant growth room.

21. Moisture balance (Ohaus) for inoculant moisture testing.

22. Assorted laboratory chemicals, including microbiological media components and stains.

23. Sieves for soil screening.

Detailed plans and equipment specifications need more time than the team had available. Arrangements should be made by EMCIP for such plans to be develcped with the involvement of a competent TDY-BNF scientist.


Fume Hood
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Laboratory Bench
Large Double Sink
New Door
Lab Bench
Analytical Balance Table
Laboratory Bench
Re-frigerator
Aisles wide enough for Lab Cart
Partitions or Cabinets
Pench
Bench
Microscopes
Shakers
Micro hood
Refrigerator
Incubater
Incandescent lights
Fluorescent lights
Small Sink
Centri-fuge
Small Sink
Spec. 20
Water Bath

BNF ACTIVITIES IN THE RICE PROGRAM

In addition to the Egyptian work on rice production in the Ministry of Agriculture and in the Faculties of Agriculture at Universities, a Rice Project with the U.S. counterpart leadership provided by the University of California at Davis is helping improve rice production in Egypt. Therefore, rice production is not included in EMCIP except as it impacts in the farming systems in which the two projects will coordinate activities. Unfortunately, the co-directors of the Rice Project were not available for discussion on the likelihood of a BNF component within the project.

This BNF team suggests that a special team be brought in to work with the appropriate Egyptians to assess the potential and to determine the research, training and extension needs for BNF in rice production. Suggested members of the team could include Dr. M. N. Alaa-el-Din, ARC, MOA, Egypt; Dr. S. A. Zaky Mahmoud, Dean, Faculty of Agriculture, Ain Shams University; Dr. Wm. Rains or S. Talley, Department of Agronomy, University of California at Davis; Dr. T. Lumpkin, Department of Agronomy, University of Hawaii; and Dr. I. Watanabe, Microbiologist, International Rice Research Institute.

Suggestions for alternates would include Dr. D. Plucknett, CGIAR Secretariat, World Bank, Washington, D.C. and Dr. Al App, Boyce Thompson Institute, Cornell University, Ithaca, New York. If requested, Dr. Lloyd Frederick of this team is willing to make contacts and arrange for such a team.

APPENDIX 1.

AGROTECHNOLOGIES BASED ON SYMBIOTIC SYSTEMS THAT FIX NITROGEN

Dr. John Halliday, NifTAL Project Director,
University of Hawaii*

Introduction

Beans and peas are well-known examples of food products from the array of plant species that belong to the legume family. Legumes are especially attractive when sustained productivity is sought from low-input farming systems. This is because of their unusual ability to be self-sufficient for nitrogen supply.

Nitrogen is an essential component of all life forms, being a cornerstone in the chemical structure of proteins. Ironically nitrogen is abundant in the atmosphere and the air we breathe is 80% nitrogen. In its gaseous form, however, nitrogen occurs as dinitrogen molecules, each having two nitrogen atoms joined by a triple bond. This is among the most stable, inert molecules known and cannot be utilized directly. Thus life on earth is totally dependent on transformations of atmospheric nitrogen to a form in which it can be used readily by plants, and subsequently, by animals and man. This process is referred to as "nitrogen fixation" and involves splitting dinitrogen into two nitrogen atoms which are then reacted with hydrogen (generated by splitting water molecules) to form firstly ammonia and subsequently a range of nitrogenous compounds. Nitrogen fixation can be accomplished industrially, but the process is one of the most energy demanding in today's agriculture. The energy cost of fixing nitrogen in the form of urea, ammonium sulphate or ammonium nitrate is compounded by the additional costs involved in its transport and application. Additionally, the rather small proportion of N-fertilizer actually taken up by the crop to which it is applied and the serious environmental pollution that can be caused by nitrogen lost from agricultural land through run-off are incentives for appraising alternate N-sources. Self sufficiency for nitrogen supply as exemplified by the legumes is thus a highly desirable trait.

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*Prepared for USAID, January, 1981.

The biological nitrogen fixation (BNF) process

Biological nitrogen fixation (BNF) in legumes is possible by virtue of the mutually beneficial association (symbiosis) which can form between leguminous plants and certain micro-organisms from a specific family of soil bacteria known as Rhizobium. Rhizobia can penetrate the roots of legumes and give rise to highly specialized organs referred to as root-nodules. These are quite different from tumors or other swellings that commonly occur on plant roots as a result of infection by disease-causing (pathogenic) organisms. The structure and function of nodulated legumes is modified in such a way that carbohydrates (sugars) produced in the leaves of the plant during photosynthesis are delivered to the nodulated root where they are respired to provide energy. In the nodules, this energy is consumed to maintain conditions under which nitrogen fixation can proceed and also to sustain the growth requirements of the rhizobia. Gaseous dinitrogen enters the nodule from the air spaces in the surrounding soil. An enzyme, nitrogenase, which is the unique contribution of the micro-symbiont, catalyses the splitting of dinitrogen molecules and the reaction of their component atoms to form ammonia. Neither the sequence of reactions and transformations that follow initial fixation nor the precise sites in the nodule where the events occur are fully understood. The steps involve very rapid incorporation of ammonia, which would ordinarily be toxic to both symbionts, into nitrogenous compounds such as amino acids, amides and/or ureides depending on the particular legume species. These are removed from the nodule in the plant's transpiration stream to be used throughout the plant as building blocks for plant proteins.

Agrotechnologies based on BNF by legumes

Most farmers in Egypt do not know that legumes fix nitrogen in their root nodules. Yet traditional and modern farming systems almost invariably include legumes. Thus legume cultivation results from recognition by farmers over many centuries that legumes are valuable components in farming systems rather than from intentional exploitation of biological nitrogen fixation per se (1,2).

Agrotechnology based on BNF by legumes has therefore two major aspects. One relates to the deliberate inclusion of legumes in cropping systems to derive benefits from their nitrogen fixation. The other concerns the intentional use of specific practices to maximize nitrogen fixation by legumes. For convenience these two facets of BNF technology will be referred to as "use of legumes" and "inoculation technology." The distinction is drawn to emphasize that currently legumes are utilized widely with less than maximal benefits from BNF because

of deficient symbiotic associations. Productivity could be increased through use of appropriate technology to assure effective symbiotic nitrogen fixation by legumes in the role they currently play in the agricultural sector. Much greater gains in productivity and economies of energy from reduced fertilizer requirements will be realized through innovative use of legumes in roles they have not occupied previously in production systems, e.g., the use of fast-growing leguminous trees in agroforestry systems. Production gains will be greatest if the use of legumes is always complemented by appropriate inoculant technology. This is because legumes can only benefit fully from biological nitrogen fixation if they encounter rhizobia with which they are genetically compatible, so that root infection and nodulation can occur and if the strain which forms nodules functions effectively in fixing nitrogen.

The use of legumes

The benefits from BNF through the use of legumes in farming systems are both direct, because the legume has an intrinsic value, and indirect, as inclusion of a legume affords greater yield stability in adverse growth conditions and can benefit companion or following non-leguminous crops. Direct benefits from biological nitrogen fixation by legumes in cropping systems arise from the multiple uses of plants in the legume family. Though known primarily for grain, forage or feed production, legumes are also cultivated in the tropics for timber, fuelwood, green manures, oils, fibers, gums, drugs, dyes, and resins. Additionally they may be used as hedges, ground covers for weed, insect and disease control, as soil stabilizers on terraced slopes or simply for shade or as ornamentals (3).

Indirect benefits accrue from the stability of performance and assurance of some economic return for at least one component under unfavorable conditions when legumes are intercropped with other crops. Stability is afforded, for example, in erratic rainfall zones when the components in the intercropping system are separated in time such as with sorghum/pigeon pea and groundnut/cotton (4,5). When there is an outbreak of pests or diseases, maize/beans and other intercrops afford stability of yields and income (6,7). Other indirect benefits accrue from the ability of legumes to make a net contribution of nitrogen to the soil under some circumstances thereby reducing the N-fertilizer requirement for a companion or following non-leguminous crop.

Inoculant technology

There is a commonly held view (8) that tropical legumes are much more promiscuous than temperate legumes in that they nodulate freely with a wide range of tropical rhizobia; and that tropical soils are laden with such bacteria to an extent which virtually guarantees effective nodulation without inoculation (9,10). This view is no longer well-founded. Some species and accessions from genera previously considered to be promiscuous (8) require specific strains of Rhizobium (11, 12, 13) or form highly effective symbioses with only a few out of the wide array of strains with which they nodulate (14, 15, 16). Recent intensification of interest in the tropical legumes and their rhizobia is revealing much greater variation in genetic compatibility and nitrogen fixation effectiveness among tropical rhizobia than has generally been acknowledged (17, 18). A plea has been made for recognition that tropical legumes fall into one of three categories (18):

Promiscuous effective (PE) group in which nodulation occurs with a wide array of rhizobia isolated from many legume genera and the resultant symbioses are predominantly effective in nitrogen fixation.

Promiscuous ineffective (PI) group in which nodulation occurs with an array of strains of rhizobia isolated from many legume genera, but in which fully effective symbioses form with only a few of those strains.

Specific (S) group in which only those strains from the same genus (or, commonly, a restricted number of other genera) form effective symbioses.

Just as with the temperate legumes, the likelihood that compatible effective rhizobia will not always be encountered already present in sufficient numbers in the soil microflora is the basis for inoculation technology for tropical legumes (19) When a tropical legume seed is sown uninoculated in a tropical soil, a native rhizobial population of strains differing greatly in their symbiotic effectiveness compete for the finite number of nodulation sizes on the legume roots. Many forage legumes bear only 10-20 nodules on which they depend for nitrogen during the first three months of their establishment. Thus, it becomes critically important that each of the nodules which form on the root contain a strain of Rhizobium that is fully effective in fixing nitrogen. The underlying objective in inoculation technology is to introduce sufficiently high numbers of preselected strains of rhizobia into the vicinity of the emerging root that they have a competitive advantage over any indigenous soil strains of lesser N-fixing ability in the formation of root-nodules.

Inoculation technology involves: selection of strains of rhizobia that are compatible and effective N-fixers with particular legumes; multiplying selected strains to high population densities in bulk cultures; incorporating the liquid rhizobial cultures into a carrier material (usually finely milled peat) for packaging and distribution; and finally, coating the seeds of legumes with the carrier or implanting the soil with the inoculant directly into the seed drill (20, 21, 16).

An inoculum strain of Rhizobium recommended for a particular host must be able to form effective N-fixing nodules with that host under a wide range of field conditions. Nitrogen fixation effectiveness is only one important criterion for an inoculant strain. Other criteria include: competitiveness in nodule formation, particularly against less effective strains; persistence in the soil in the absence of the host, especially for strains for annual species; promptness to form nodules; ability to fix nitrogen under a range of soil temperature conditions; tolerance to pesticides; tolerance of low soil pH; nodulation in the presence of high levels of soil nitrogen; and ability to grow and survive in peat inoculants.

The host genotype interacts with the infecting strain of Rhizobium in determining the level of nitrogen fixation with the host playing the dominant role. Thus two sources of variation (plant and Rhizobium strain) can be exploited in selection programs. Most commonly, though, the plant is selected independently and a suitable strain sought thereafter, thus allowing only for exploitation of strain variability. The range of specificities of host genotype interactions is well illustrated by soybean (22) and in the African clovers (23).

Such specificities give three options in the approach to selection of strains for inoculants: numerous inoculants, each with a highly effective strain for individual species; 'wide-spectrum' strains that vary from good to excellent in N_2 fixation with a range of legumes; or multiple-strain inoculants containing the best strain for each host species. There may be a conflict between the option that would be chosen for commercial expediency and that which is scientifically excellent (24). In Australia 'wide-spectrum' strains are used when these are available, but there is increasing use of specialized inoculants with specific strains for individual hosts. Despite findings which suggest that multi-strain inoculant should be avoided because of possible antagonistic and competitive effects in culture (25) this is the approach used successfully by the U.S. inoculant industry.

Strains for testing can be obtained from other laboratories working with the same species, from nodules on plants in the native habitat from which they were originally collected for plant-introduction/evaluation programs, and from nodules formed on the legume by native strains after sowing uninoculated seed in the region where the new species is expected to be used. None of these sources is invariably better than the other in screening programs.

Most legume inoculants are prepared by adding liquid cultures of Rhizobium to a finely-ground carrier base material such as peat. Although mixtures of peat with soil or compost mixtures, lignite, coir dust and some other organic materials have been used, peat has proven to be the most acceptable carrier world-wide. Agar, broth and lyophylized cultures are not recommended because of the very poor survival of these forms of the inoculum on seed (27, 28, 29).

Peat cultures can be prepared in two ways. Either ground (milled) peat is mixed with a high viable count (more than 10^9 rhizobia/ml) broth culture in sufficient volume to provide the minimum number of Rhizobium acceptable for use, or sterilized peat is inoculated with a small volume of culture and incubated to allow multiplication of the rhizobia in the carrier. The choice of method will depend on two main factors--the survival of the rhizobia in peat in numbers high enough to meet a minimum standard of quality, and the availability of suitable, sterilizable containers and sterilizing facilities. The two factors that most affect survival of rhizobia in peat are temperature of storage and sterility of the peat. There are differences among species and also between strains of the same species of Rhizobium in their ability to survive well in peat (30).

Like all biological products, legume inoculants are prone to loss of quality owing to variation in the organism concerned and from unforeseen factors affecting some aspect of growth or survival. It is therefore essential that a quality control system be established. In Australia large-scale manufacture of legume inoculants is by private enterprise, and a separate, official (government) control laboratory is responsible for maintaining a high quality product. The control laboratory maintains and supplies recommended strains of Rhizobium to the industry, checks strains annually for ability to fix N_2, assesses quality of cultures during and after manufacture, and conducts such research as may be necessary to overcome problems associated with production and survival in the final product. In the U.S. the industry is free to select its own strains and official control ensures that the product can form nodules on the legume for which it is recommended.

Although control of quality of inoculants is primarily in the manufacturer's interest and therefore his responsibility, power of control by external bodies provides protection from less scrupulous operators and genuine failure of a strain outside manufacturer control. Not all countries back their control labs with legislation. A control group requires suitably qualified and experienced personnel with facilities to permit normal aseptic culture transfer and plant growth facilities suitable for legumes over the range for which inoculants are manufactured. Methods of assessment involve both qualitative and quantitative tests. The number and extent of these may vary according to competence and experience of manufacturers and the standards desired. In Australia this control extends to holding stocks of the strains used in inoculants. This is not the case in the U.S. (13, 31). In addition to assessment of quality throughout manufacture, it is important to monitor quality of product in retail outlets. Standards acceptable at this level may vary from that at manufacture and between countries. It is important that standards be realistic and within the capability of manufacturers yet ensure that sufficient viable rhizobia are applied to the seed to provide a satisfactory inoculation. In many instances this can be as few as 100 rhizobia per seed but in case of severe invironmental stress as high as 10,000 or even 500,000 (32, 33, 28).

The prime objective of inoculation of legume seed with rhizobial inoculants is nodulation of the introduced legume host plant. Rhizobia introduced into new environments must live saprophytically in competition with other rhizobia and soil microorganisms in an environment which may be adverse for their growth and survival, until such time as the host seedling roots provide the ecological niche to which they are adapted. Thus steps should be taken to ensure that inoculant strains: remain viable until the host seedling is at the susceptible stage for infection; compete with any naturalized rhizobia for infection sites on the roots of the host legume and so form sufficient nodule tissue to permit maximum nitrogen fixation; nodulate its host promptly and effectively over a range of environmental conditions; and persist in the soil for at least several years in sufficient numbers to maintain nodulation of perennial legumes or to achieve prompt nodulation of regenerating annual species.

The first attempts at inoculation involved the transfer of soil from one field to the next, but with the isolation of the organisms responsible for nodule formation, artificial cultures soon replaced the laborious soil transfer technique. The usual inoculation technique is to apply the inoculant to the seed just before sowing either as a dust or as a slurry with water or adhesive solution. Adhesives such as gum arabic and

substituted celluloses not only ensure that all the inoculum adheres to the seed surface but also provides a more favorable environment for survival of the inoculum. Pelleting of seed with finely ground coating materials such as lime, bentonite, rock phosphate and even bauxite (32, 34) have been used to protect rhizobia during their time on the seed coat. Pelleting is a simple on-farm technique (32, 35) but custom-pelleted (by seedsmen at farmer's request) and preinoculated seed is now more popular. This latter procedure is potentially able to provide high populations of rhizobia on the seed for long periods of time (one growing season to the next) but has not yet been fully developed or exploited. Most preinoculation procedures are based on multiple coatings, alternately of adhesive and finely ground pelleting materials as used in simple pelleting. The peat inoculant is included as one (or more) of these coating layers. Soaking seeds in a broth suspension and then exposing them to either high pressure or vacuum to impregnate the rhizobia into or below the seed coat has not proven successful. Theoretically, rhizobia introduced in this way would be protected from drying and other adverse environmental conditions, but the quality of products produced commercially has been variable to very poor (36, 37, 38). It is, in fact, an indictment of the research workers in this area that 25 years has yielded so little progress in an area that has so much to offer for those concerned with the practical aspects of agricultural microbiology. The technique is particularly applicable to the less well developed and inexperienced rural groups in that a high quality and reliable product could be marketed by a manufacturer or seeds distributor thus eliminating the need for farmer involvement in legume inoculation.

An alternative to pelleting and preinoculation in recent years has been the use of concentrated liquid or solid granular peat culture which can be sprayed or drilled directly into the soil with the seed during planting. Suspensions of rhizobia either as reconstituted frozen concentrates or suspensions of peat inoculant can be applied with conventional equipment. Similarly, granulated peat inoculants can be drilled in from separate hoppers on the drilling equipment. These methods have been especially successful for introducing inoculant strains into situations where there are large populations of competing naturally occurring soil rhizobia (39) or in cases of adverse conditions such as hot-dry soils (40) and where insecticide or fungicide seed treatment precludes direct seed inoculation (41, 33). Solid inoculant, also known as granular or "soil implant" inoculum, is advantageous also, where seeding rates for crop legumes of 70-100 kg/ha make on-the-farm inoculation logistically impracticable.

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Current use of legume-based BNF technology

(a) The use of legumes: The grain legumes are cultivated widely in a variety of agro-climatic zones in the tropics and subtropics. Total area in grain legumes in 1979 was 175 billion hectares. Dry bean (Phaseolus vulgaris) is the most important grain legume in Latin America, groundnut (Arachis hypogaea) in Africa and collectively groundnut, pigeon pea (Cajanus cajan) and chickpea (Cicer arietinum) in Asia. These and other grain legumes have been consistent components of human diet in the tropics for centuries yet in quantitative terms they continue to be minor crops.

The use of legumes in mixed legume/grass pastures in the tropics is at present restricted to northern Australia, the United States (Hawaii, Florida), southern Brazil and northeastern Argentina. The total area in improved legume/grass pasture is insignificant compared to the area of native grasslands under grazing. The use of temperate forage legumes in mixed pastures at high altitude locations in developing countries within the tropics is feasible but is considered outside the scope of this report.

Production statistics for the tropical grain legumes are seldom accurate. Most of the production is on a subsistence scale on small farms and the yields are seldom included in official statistics. Thus a figure of 186 million tons (42) should be regarded as an understatement.

There are very many agencies supporting and conducting research related to the use of legumes. International agencies such as FAO, UNDP, IBPGR and the IARCs all have grain and forage legume programs. The USAID together with the governmental agencies of many countries engaged in foreign agricultural development support research on legumes. The World Bank and several private and public foundations also support legume research. The author is not aware of any country in the tropics that does not have a legume project within its official agricultural program. Additionally, universities and agricultural colleges in tropical countries usually have legume programs. These projects cover the physiology, plant nutrition, agronomy, pathology, entomology breeding and seed production of legume crops. Insofar as BNF proceeds at a rate governed strongly by the plant's ability to deliver carbohydrate to its root nodules, most technologies that improve overall plant performance are likely to have a beneficial impact on nodulation and nitrogen fixation. Relatively few projects, however, give adequate attention to specific techniques for maximizing biological nitrogen fixation by the respective legume. In fact some research programs with legumes are conducted under nitrogen-fertilized conditions or in fertile, N-rich soils. Breeding for high-yielding varieties under such

conditions has resulted in plant types which are only weakly symbiotic and heavily dependent on soil nitrogen; e.g. bush bean (Phaseolus vulgaris) varieties developed in the United States.

Given the important role of grain legumes as the major dietary protein source for low-income groups in the developing countries, it is hardly surprising that such a multitude of funding agencies and implementing organizations give attention to research on legume technology. Whilst it is to be expected that there will be overall gains in the quantities of nitrogen fixed from improved performance by legumes in the roles, and on the acreage they currently occupy, the major gains in BNF through the use of legumes will follow increases in the total land area in which legumes are grown and especially the innovative use of hitherto underutilized legumes.

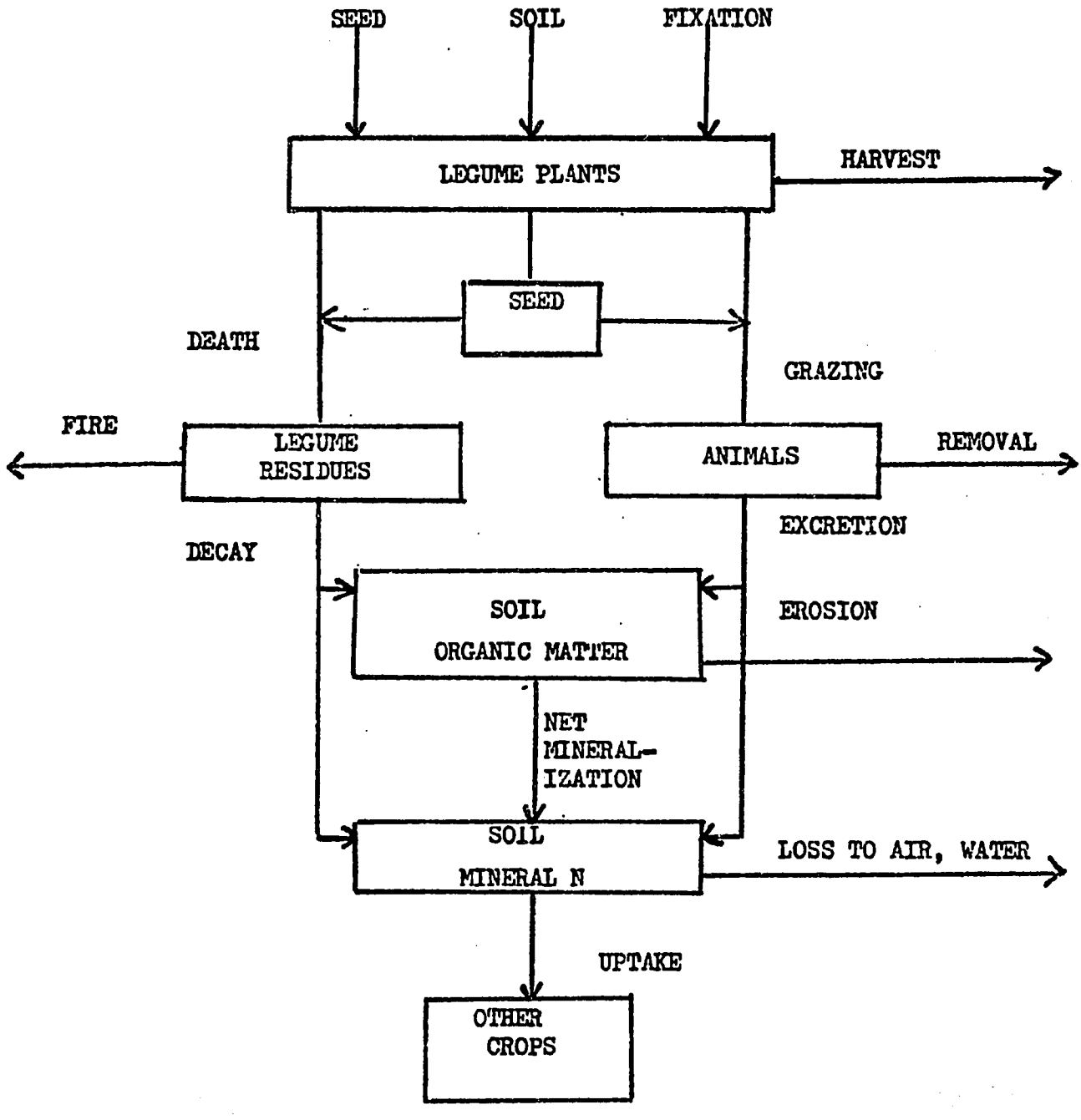
(b) Inoculant technology: Inoculant technology is implemented widely on a commercial scale mainly in the developed countries, the United States and Australia having substantial industries for the production, distribution and marketing of legume inoculants. There is also commercial-scale production in Brazil, Uruguay, Argentina, India and Egypt. Inoculants are available commercially in many other countries but produced in U.S. or Australian laboratories for importation and marketing by a subsidiary of the parent company or suppliers of legume seed, e.g. the Nitragin Company, Milwaukee, has subsidiaries in Mexico, Venezuela and Argentina and distributes inoculants worldwide. Some research centers, such as CIAT and the University of Hawaii NifTAL Project produce inoculants in pilot-scale plants as a service mainly to researchers and occasionally to legume growers. Demands for inoculation technology are increasing, associated mainly with the increased use of soybeans. There are dangers in trying to satisfy this demand by importation of inoculants developed in the U.S. or elsewhere. This is because inoculation technology as presently practiced has not proven transferable. That is to say, strains of Rhizobium and inoculation methods developed for conditions at one location in a particular farming system do not perform equally well at another location in a different farming system. Furthermore, the viability of rhizobia in legume inoculants is greatly affected by storage conditions during shipment. Since producers are unable to control such factors, no guarantee can be given that the inoculants are of merchantable quality on arrival at their destination. For both these reasons inoculation failures are a common occurrence and this is affecting adversely consumer acceptance of the technology. An ideal scenario for improved implementation of BNF technology is described in a later section.

Those organizations funding research to adapt inovulant technology to the circumstances in which it will be used in tropical countries are: UNDP by its support to the IARC's through CGIAR and for a-specific research program involving IITA and BTI/Cornell University; UNEP and UNESCO support inoculant technology under the MIRCEN project- FAO is actively considering the role it might play in the adaptation of inoculant technology of use in developing country agriculture (consultancy reports have been prepared and a planning workshop held during 1980); USAID through its contracts with University of Hawaii (NifTAL Project) and USDA, Beltsville ARC Section 211-(d) to the U.S. Universities' Consortium on BNF in the tropics, and through a portfolio of small grants administered by USDA SEA/CR; USAID and several governmental and non-governmental agencies who support the CGIAR are thereby sponsoring work at CIAT, IITA, ICRISAT and ICARDA on the adaptation of inoculant technology for use in the tropics.

How BNF by legumes increases crop yields and soil fertility

Consideration of the possible pathways for transfer of N from legumes to other crops (Fig. 1) affords a model around which estimates of the relative importance of the pathways of transfer of N from legumes to other crops and/or the soil can be attempted. Nitrogen gains per hectare per year entering the cycle as seeds, 1-2 kg (43) and in acid rainfall, 1.5 - 3.5 kg (44) are small compared to the nitrogen accumulated from the soil fixed biologically. About 50% of the nitrogen accumulated in legumes in fertile soils is attributed to BNF (45) though the proportion from fixed nitrogen will be greater in impoverished soils and lesser under nitrogen fertilization. Nitrogen accumulation in legume monocrops ranges from 50 - 100 kg/ha/year. It is generally accepted that N fixation of around 100 kg/ha can be expected from the majority of grain and forage legumes. High levels are typically those of leucaena and other forage legumes with a twelve-month growing season. Low levels are typically those of notoriously bad nitrogen fixers with short growing seasons (e.g. Phaseolus vulgaris). As an illustration, the fate of, say, 100 kg of biologically fixed N entering the cycle will now be followed. Between 60 - 90% of the nitrogen accumulated in legumes is removed as grain depending on the species, harvest index and harvesting practice, or as animal products depending on the intensity and selectivity of grazing. Thus in an intercropping system only 10 - 40 kg nitrogen could potentially benefit other crops. Some of the organic nitrogen of the legume residues is mineralized rapidly and the rest is added to the soil organic matter pool from which it is mineralized slowly over a much longer period. In the studies that have been performed, 60% is probably the maximum portion of the nitrogen in the organic residue of a legume crop that could be mineralized in time to benefit a following crop.

Figure 1. Pathways for flow of N from legumes to other crops (from Henzell, 1977)



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Using 50% initial mineralization for the purposes of these calculations, in a cropping system in which the legume fixes 100 kg/ha/year only 5 - 20 kg of nitrogen is likely to benefit the following crop. One practice that would substantially increase the contribution is green manuring. On the basis of the level of nitrogen fixation cited above (100 kg/ha/year), a residual benefit of 50 kg/ha/year to a following crop could be anticipated after incorporation of one year's production into the same area prior to raising the following crop. Greater benefits still can be contemplated if the green manure from a large production area is carried for incorporation into a smaller crop production area. Experience has shown, however, that crops do not necessarily respond to exaggerated applications of green manures. There are few farming systems in which green manuring is economically feasible (43, 46) since land is tied up without immediate economic return. Where green manuring is practiced, 5 tons of green matter per hectare is an accepted application rate (47). This would represent an addition of only 40 kg/ha of nitrogen to the soil, of which only about 20 kg would mineralize to the benefit of the crop. Real data, though limited, supports such an estimate. Green gram contributed 22 kg of nitrogen to following crops and calapo/stylo green manure contributed 15 kg (48).

Moving on to consider the nitrogen economy of mixed cropping systems in which the legume and non-legume are growing concurrently, the situation is more complex. Legumes usually take up less soil nitrogen in competition with non-legumes and a greater fraction of the nitrogen they accumulate in mixed crop is from fixed nitrogen. Somewhat surprisingly the nitrogen fixation of intercropped beans (Phaseolus vulgaris) per hectare is not significantly different from beans raised in monoculture (49). This is attributed to competition between the maize and the bean for light and nutrients beginning after the decline in nitrogen fixing activity in the root nodules of the beans. Not all legumes shut down nodule function as early in the growth cycle as Phaseolus vulgaris and the effect of inter-cropping on nitrogen fixation may be detrimental in other intercropping systems.

It is a common misconception that there is substantial direct transfer of nitrogen from the legume to a non-legume companion species in a mixed cropping system. There is no convincing evidence that actively growing, healthy legumes, whether grain or forage, excrete significant amounts of nitrogen from their roots or nodules.

The hypothesis originally proposed by Virtanen and co-workers (50,51,52) that surface excretion of simple amino compounds from healthy, functioning legume root-nodules resulted in direct transfer of significant quantities of nitrogen to non-legume companion species has found little support from other workers (53,54,55,56,57).

Subsequent research under carefully controlled conditions using the 'fog box' technique (58) indicated that excretion of a wide range of substances from plant roots does occur, but that the quantities involved are small, being less than 0.5% of the plant's nitrogen (59). Stated differently, a crop fixing 100 kg of nitrogen a year would excrete only 0.5 kg to the soil.

Nitrogen benefit to non-leguminous crops through association with companion legume species is considered to be of an indirect nature through loss and decay of shoot, root and nodule tissue, or by recycling via the grazing animal, rather than by a direct pathway (60,61,62,63).

Clearly then, mixed cropping systems that aim to utilize legume-fixed nitrogen for the benefit of a companion non-legume species must match species such that the non-legume is longer-lived than the legume because nitrogen will be released in significant amounts only after cessation of active growth and decomposition of tissues of the legume. The maize/bean association used widely in Latin America exemplified this principal. Estimates place fixation by beans at 20 - 40 kg of nitrogen per growing cycle (64). Assuming 70% removal of nitrogen as protein in the legume grain, this leaves only 6 - 12 kg in legume residues of which 3 - 6 kg (assuming 50% mineralization) will be mineralized in time to benefit the maize. Some estimates place the mineralization that can benefit a companion species as low as 20% and consistent with this it is not uncommon for there to be no detectable nitrogen benefit in companion crops that are inter cropped with legumes.

From the preceding quantitative considerations, it is clear that the biological nitrogen fixation benefit to non-legumes due to inclusion of legumes in a cropping system is small indeed compared to the level of nitrogenous fertilizer use in the more intensive cereal production systems of the developed world. Thus the principal contribution of biological nitrogen fixation to human nutrition will continue to be via the protein in legume [illegible]. Important implication of the data discussed here is that the suggestion of substantial replacement of nitrogen fertilization of cereals and root crops by biologically fixed nitrogen is unrealistic as these crops are known to respond to levels of nitrogen fertilizer far in excess of those which could currently be supplied through BNF by legumes. Thus there is an urgent need to devise ways of increasing the contribution which

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BNF by legumes can make to cropping systems as a complement to N fertilizer-based production rather than as an alternative to it.

Legumes can be managed to increase their nitrogen contribution to companion or following crops. They vary in total nitrogen fixed, the proportion retained in non-harvested residues, the percentage nitrogen level in residual tissue and the facility with which the organic nitrogen is mineralized. The greater are these parameters, the greater will be the residual nitrogen benefit. Given this situation, the priority given in legume breeding programs to improving their harvest index, i.e. maximizing the fraction of each plant's total production that is removed as grain, should be called into question.

In summary, it can be said that the principal benefits from BNF through the use of legumes in farming systems of the tropics are derived from the dietary protein of the legume grain, the multiple uses which legumes serve for the subsistence farmer, and the greater stability of yield and financial return of intercrops over monocrops. The indirect benefits from contribution of biologically fixed nitrogen to companion or following species are small but are significant at present in the context of input levels in subsistence farming in the tropics.

At present there is insufficient reliable data on the benefit to be derived from enhancing, through inoculant technology, the nitrogen fixation in tropical legumes over and above the level which would be anticipated from spontaneous nodulation with native strains already present in the soil to advocate that rhizobial inoculation always be performed. It is tempting to recommend rhizobial inoculation of all legume sowings as an insurance measure against the risk of nodulation failures that would otherwise occur. However, inoculant technology does represent a cost, albeit small, and does add a degree of complexity to the sowing practice. Thus inoculant technology should only be advocated when there is known to be a need-to-inoculate and a demonstrable benefit therefrom. Additionally the concept and practice of inoculant technology is so foreign to farmers' normal practices that it should not be recommended lightly. A subsistence farmer can be forgiven for not comprehending nor accepting a technology that involves sticking black powder containing bacteria to his seeds. This contradicts concepts about which he has only recently become educated, namely, that bacteria are bad and clean seed is important. It is to be questioned whether inoculant technology in this form will ever be accepted widely among subsistence farmers in the tropics and subtropics. Further discussion of this topic follows later.

Unfortunately many trials performed to evaluate inoculant technology with tropical legumes under tropical conditions have been performed with imported inoculants which may not have contained acceptable levels of viable rhizobia at the time of use. Lack of response to inoculation in such trials does not preclude the possibility that the legume could potentially benefit from inoculation. More recently coordinated networks of trials have been initiated to determine whether there is an economic yield benefit from inoculation of legumes or not. INTSOY conducts international Soybean Rhizobium Inoculation Experiments (ISRIE) throughout the tropics. CIAT distributes an International Bean Inoculation Trial (IBIT) throughout Latin America. The University of Hawaii coordinates an Internatinal Network of Legume Inoculation Trials (INLIT) offered for 13 agriculturally important legumes and involving a 3-stage experimental program in which cooperators throughout the tropics select strains specifically for their legume variety and local soil conditions thereby maximizing the opportunity for a yield response following inoculation.

Future potential of legume-based BNF technology

Despite their seeming attractiveness for sustained productivity from low-input production systems, and despite also their consistent strategic use in many farming systems,legumes often have remained minor crops in the systems in which they occur (65).

Why is this the case, and what factors would lead to greater use of nitrogen fixed biologically by legumes? A small-scale, subsistence farmer elects to raise those crops that best meet the various needs of his household but also chooses onecrop, at least, to sell or exchange for goods or services for which he is dependent on others. Large-scale farmers consider primarily the economic return and facility of management associated with the crops they will choose to plant. A grower preference for cereals over legumes, when the grain is to be marketed, would be understandable. It is usual for yields of cereal grains to be as much as four times higher than legumes (typically 3.0 t/ha vs. 0.7 t/ha). Although the protein content is much higher in legumes (30%) than in cereals (6%) the market value of legume grains, albeit higher than for cereals, does not compensate the grower for their low relative yield.

Many factors will contribute to an increase in the use of [illegible]ls will continue to be the major source of protein [illegible]r human nutrition on a worldwide basis but an increase in importance of root and tuber crops and plantains over the next two or three decades is anticipated (66). Legumes can be expected to be one means of complementing the diet quality of these starchy foods that are deficient in protein.

Another factor that has already caused a re-appraisal of biological nitrogen fixation through legumes is the cost and availability of energy for production of nitrogen fertilizers. Already 20% of N fertilizer production in the U.S. is cost-ineffective because of the cost of energy (in the form of natural gas) for the process. Producer costs have been calculated as $160 per ton (67) whereas the selling price is in the range of $85 - $105 per ton. It is predicted that by as early as 1984, 60% of the world's ammonia production will be by the USSR, Eastern Europe, and the developing countries, and that ultimately N-fertilizer production will be economically feasible only in those countries with huge surpluses of natural gas (mainly Mexico and Indonesia).

Thus biological nitrogen fixation through the use of legumes may be resorted to increasingly, not only to reduce the cost of on-farm inputs, but also to save foreign exchange and avoid over-dependence on foreign powers.

But economic pressure alone will not guarantee adoption of biological nitrogen fixation-based technology without compelling demonstration of greater benefits from BNF by legumes than are currently recognized. The dramatic increase in interest in BNF since the energy crises of 1973/4 and 1979 has brought it under the scrutiny of agencies and individuals whose concern is its viability as a productive agricultural technology now rather than its often acclaimed potentials for the future. This should encourage the agricultural research community to undertake a comprehensive program of technology development in which the relative distribution of funding and manpower investment is realistically prioritized. Thus research aimed at stabilizing grain legume yields is likely to increase the contribution of biological nitrogen fixation in tropical farming systems to a greater extent than much of the research on the BNF process per se in grain legumes. Similarly, research involving selection of forage legume germplasm that is adapted to the soils and climates of the world's under-utilized savannahs, and development of appropriate legume-based pasture management and utilization technology can be expected to bring about a major increase in the use of biological nitrogen fixation even without further increase on the BNF process per se in those legumes. These statements assume of course that those BNF components of each legume production package that are acknowledged to be indispensable can be guaranteed, i.e. effective nodulation. Since this is not always the case, those specific aspects of BNF research which tackle the factors which limit nodulation and nitrogen fixation by legumes in tropical soils should be singled out and given highest priority. Unless pursued concurrently and with an intensity that assures that outputs on the BNF component for each legume are in synchrony with other advances in the use of those legumes, peformance of the legume "package" will be seriously impaired.

Constraints to implementation of BNF Technology

There are still many unknowns in the scientific understanding of BNF, and research into the biochemistry and genetics of the process is particularly intense and competitive.

But few, if any, of these unknowns are really constraining the implementation of legume-based BNF technology. The basic principles of inoculant technology have been known for many years and has [illegible] major contributions to agricultural production initially in Australia and, more recently, worldwide as soybean cultivation has been increasing. The real constraints to fuller implementation of BNF technology relate to delivery of the technology, both to potential inoculant producers and to farmers, and acceptability of the technology, again by both the inoculant producers and farmers.

There has not been adequate demonstration, under realistic, farm-relevant conditions in the developing countries, of the yield increases and/or reduced fertilizer needs that are repeatedly stated to be the benefits of BNF technology. In some cases, inoculation trials have been performed and no response obtained. But these trials have been mainly with imported inoculants, the quality of which at the time of their use was not or could not be verified. Thus a related constraint is the lack of trained personnel with the essential combination of agronomic and microbiological skills for executing production-oriented research on BNF techology. Such research is necessary for adaptation of BNF technology including development of appropriate Rhizobium strains and inoculation procedures for use under the variety of circumstances encountered in the tropics and subtropics. It must be remembered that current inoculation technology as used in the U.S. and Australia is suited to legumes grown under favorable conditions with relatively high complementary agronomic inputs. Transferability of this technoloy to situations where the legumes are grown usually in marginal conditions with minimal inputs, and confronted with one or more soil and climatic stresses is in some doubt (68).

It is the genotype of the legume which is to be inoculated that is the prime determinant of the strain used in rhizobial inoculants, rather than the characteristics of the soil into which [illegible] introduced. This is contrary to what is expe[illegible] time users of inoculants.

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For example, in providing inoculant services in Latin America and Hawaii, it has been common to receive data from soil analysis together with requests for inoculants. This illustrates that farmers expect that the election of legume inoculant is made after consideration of local soil and climate, just as would be the choice of crop variety. Yet there is only one instance in which an inoculant strain recommendation for an inoculant in commercial production takes into consideration the soil characteristics into which it will be introduced. Rhizobium strain CB 81 is recommended for Leucaena leucocephala sown in acid soil and NGR 8 for alkaline soils (69).

When soil characteristics are very different, the response to inoculation and the relative performance of rhizobial strains is also different. Even when soils were selected for their apparent similarity, the performance of rhizobia in those soils was very different. It is not surprising therefore that some authors have advocated that simple "need-to-inoculate" trials always be performed at the local level due to the unpredictability of the response to inoculation (32,16,18). If carried to its conclusion, this suggestion would result in legume inoculation being tested, essentially by trial-and-error, at every site where legumes are to be grown. It is certainly to be hoped that inoculation technology is more transferable than this, otherwise its value as an agrotechnology is questionable.

It is well known that there are significant differences between sites in the size of their indigenous rhizobial populations (70, 71) and in the range of strains of Rhizobium in the indigenous microflora (72,71). Such differences have been atrributed to the effect of soil factors (72,73) though the possibility of widespread correlations between specific soil characteristics and rhizobial occurrence in tropical soils has not been critically examined.

It is also acknowledged that the response by tropical legumes to inoculation with rhizobia varies from site to site (32,16,74,75, 15). Such variation has been attributed to: differences in number, effectiveness and competitiveness of native strains (76, 77,71,17); variation in quality of the inoculant at its time of use (78); and to variation in soil nitrate levels (79). The possibility that the response to inoculation could be predicted on the basis of a more thorough description of soil and environmental characteristics has not been tested.

BNF technology is a difficult technology to deliver by normal extension mechanisms. Thus a lack of illustrative and explanatory pamphlets and other aids both for extension agents and the farmers with whom they have contact is also a constraint on implementation of BNF technology at the farm level.

Furthermore, few of the senior administrators and decision makers who determine agricultural policy in the developing countries are fully aware of the applications for legume-based BNF technology in the agriculture section of their countries. Most policy makers are aware of some of the attributes of legumes. Relatively few of those individuals appreciate the role played by biological nitrogen fixation in legumes and among those few an even smaller proportion recognizes that it may be essential to employ specific technologies to ensure that nitrogen fixation occurs at all, let alone at a maximal rate. Thus there is a need for educational material, specifically developed for this clientele group, bringing to their attention the real need to adapt currently available technology to the particular circumstances in which it is to be employed in their country.

As BNF technology is being implemented, new constraints are emerging that are best described as "scientific" and are researchable. For example, some countries do not have peat deposits suitable for carrier materials for inoculant production and alternate materials must be identified and validated. Also, specific soil and climatological stresses such as extreme soil acidity and the associated high levels of toxic elements like aluminum and manganese may require selection of strains of rhizobia tolerant to those conditions for use in inoculants.

It could be suggested that the large number of researchers with the competencies required for BNF research but who expend their energies and resources researching aspects of BNF other than limiting factors such as the examples cited above is also a constraint on fuller implementation of BNF technology. Funding agencies do not always recognize a distinction between applied and less useful research that competes for resources under the general subject matter area of biological nitrogen fixation. Biological nitrogen fixation has great pertinence to agriculture production in developing countries but not all research conducted under the BNF umbrella is applicable in agriculture.

It is also known that the relative performance of strains selected under optimal conditions for a specific legume is variable, depending on the site to which they are introduced (15). With inoculants that contain a mixture of strains of Rhizobium, it is common for one strain to dominate in the nodule population that results from the inoculation (80,81). The possibility that rhizobial strains might be selected for adaptation to particular soil and environmental conditions is not presently exploited in tropical agriculture.

A serious constraint to fuller implementation of BNF technology is non-availability of domestically-produced, high quality inoculants within each of the countries of the tropics and sub-tropics. Thus factors which deter government organizations or private enterprise from undertaking inoculant production in a particular country are also constraining BNF technology. Among these are: high capital cost of inoculant production plant (of type used in the U.S. and mistakenly assumed to be a prerequisite for any production plant); high operational cost associated with retaining a professional and well-trained staff to run the plant; operational risks associated with losses due to such factors as contamination; absence in most developing countries of an adequate infrastructure that would permit marketing and distribution of a biological production with notorious vulnerability to damage by high temperatures; reticence to embark on an enterprise in advance of official control standards being established (confounded by official reticence to set standards until there is an industry to be controlled); insufficient present demand and uncertain future demand for inoculants.

As has been referred to already, the present nature of the technology meets considerable farmer resistance, i.e. the coating of seeds with peat inoculant. In Brazil, packets of inoculant are included "free" by some seed distributors with all seed sales. However, the inoculant is frequently discarded by farmers not only because of the nuisance associated with use of the inoculation on a field scale, but also in part because of an unfortunate impression that if inoculant is "free" it is suspect and of little value.

The cost of inoculants is not usally a constraint to the use of inoculant by farmers who outlay capital for seed. Inoculant will [illegible] of the seed cost. For subsistence farmers who [illegible] purchase seed off-the-farm, the capital outlay for [illegible], albeit small, may be a disincentive to the use of inoculants. Cost becomes a more important consideration with granular forms of inoculant in which the rate of application is much greater than with seed-applied inoculant.

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Scenario for full implementation of BNF technology

Given that the constraints on fuller implementation of BNF technology are not solely scientific but include cultural, socio-economic and political factors, the scenario in which BNF might realize its potential would necessarily be multi-faceted and comprehensive.

The current trend toward energy-efficient farming systems to reduce capital outlay for fertilizers that must be imported can be expected to continue and intensify. Because of the high energy consumption during manufacture of nitrogen fertilizers, their price and availability is influenced increasingly by oil-rich nations. There is added attractiveness in alternate nitrogen sources to avoid even further dependence on foreign powers. Legume-based BNF technology is the major option available and is likely to be resorted to more and more. As has been described, the use of legumes and appropriate inoculant technology has the potential to increase the amount of biologically-fixed nitrogen entering agricultural production systems. Given that the main value of legumes is their high-protein grain, rather than their nitrogen contribution to non-leguminous food crops such as cereals and root crops, the scenario for full realization of BNF technology's potential would need to include a swing in consumer preferences away from crops that depend so heavily on nitrogen fertilizer. Thus, in the gambit of BNF research priorities, attention will need to be given to learning the cultural and scientific bases for these preferences and to alleviating where possible the constraints to greater consumer acceptance of legumes.

As explained earlier in this report, the major increases in benefits from legume-based BNF technology will arise through: an increase in the total acreage in legume production; innovative use of legumes in roles they have not previously occupied; and by ensuring that biological nitrogen fixation in those legumes is maximal through appropriate inoculation technology. Much remains to be done to improve the biological nitrogen fixation components in the technology package for legumes in the role they currently play in agriculture. There is a wide discrepancy between farmers' yields and the known yield potential of the grain legumes. Furthermore, it is disconcerting that in the majority of reports of legume trials that included rates of nitrogen fertilizer application, the legumes responded to nitrogen fertilization--disconcerting because this means that even when legumes were grown under favorable management in experiment stations, let alone in farmers' fields, the symbiotic association of the legume with Rhizobium was defective. There is therefore the potential to double or triple the nitrogen benefits described in this report through development of technology that would assure establishment of maximally effective rhizobial symbioses in tropical legumes under tropical conditions.

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Impacts of wide-scale implementation of BNF technology

The use of legumes to benefit other non-leguminous crops implies mixtures of rotations or crops. The production systems are therefore labor intensive and, in the case of mixed crops, not readily mechanized. Since this is already the circumstance of most production in developing countries no impact is likely. If BNF technology were to be employed in the U.S., for example, this would necessitate a swing away from the highly-mechanized monocrop systems currently employed and a major demand for farm labor.

Inoculant technology is scale-neutral but the use of legumes as green manures or in multiple crops and rotations is not. Such practices are often more suited to small farms than large farms.

Inoculant technology does not increase nor displace appreciably the demand for labor. The intentional use of legume residues for the benefit of non-leguminous crops will create demand for laborers. Since the technology involves extra cultivation steps that are not easily mechanized. Inoculant production will require a small demand for labor, and can provide an opportunity for a private enterprise.

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APPENDIX 2.

ANNUAL REPORT OF COOPERATIVE REGIONAL PROJECTS

Supported by Allotments of the Regional Research Fund
Hatch Act, as Amended August 11, 1955

January 1, to December 31, 1980

PROJECT: S-130 Associative Nitrogen Fixation in Grasses

COOPERATING AGENCIES AND PRINCIPAL LEADERS:

State Agricultural Experiment Stations	Participants
Arkansas	J. T. Gilmour*
Florida	S. C. Schank*
	R. L. Smith*
	K. H. Quesenberry*
	D. Duggan*
	D. H. Hubbell*
Georgia	J. H. Bouton*
Louisiana	E. P. Dunigan*
Nebraska	R. V. Klucas*
New Mexico	A. A. Baltensperger*
	G. O. Throneberry
Texas	D. A. Zuberer*
	R. W. Weaver

USDA/SEA

Florida	M. H. Gaskins*
	S. Albrecht
Maryland	C. Sloger*
	L. D. Owens*
	P. van Berkum*
Puerto Rico	A. Sotomayer-Rios*
Texas	B. Burson
	J. H. Everitt

COOPERATORS WITHOUT OFFICIAL COMMITMENTS:

Alabama	A. E. Hiltbold
	W. Hill
Florida	J. Gamble
	J. Milam
	N. C. Schenck
	I. K. Vasil
Georgia	G. W. Burton
Louisiana	G. Sims
New Mexico	B. A. Melton
	R. B. Turner
North Carolina	G. H. Elkan
	A. G. Wollum
South Carolina	H. D. Skipper
Texas	D. M. Vietor
	S. Wright
	L. M. Zibilske

* Voting Member

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PROGRESS OF THE WORK AND PRINCIPAL ACCOMPLISHMENTS:

1. Objective A (screening for grass-bacteria associations)

Bouton (Georgia) continued to screen pearl millet genotypes inoculated with Azospirillum sp. in axenic, N-free, seedling agar tubes. Genotypes which support high and low acetylene reduction were identified. Cores of tall fescue sod support low acetylene reduction.

Klucas (Nebraska) screened 15 lines of 'Park' Kentucky bluegrass and found no differences in rates of acetylene reduction. Activities were higher under light conditions.

Zuberer (Texas) completed isolation and characterization of bacteria from soil-root cores of grasses suporting acetylene reduction. Most isolates were Klebsiella sp. and Enterobacter sp.

Gilmour (Arkansas) utilized a visual score for the degree of colonization and the acetylene reduction assay to identify promising rice variety/bacteria combinations. The bacterial isolate and rice genotype are equally important in establishing associative N_2 fixation in the seedling stage.

Baltensperger and Throneberry (New Mexico) obtained native bacterial isolates from roots of bermuda grass for testing with bermuda grass and wheat.

Shank, Smith, and Quesenberry (Florida) screened 305 soil-plant cores from 57 counties to find grass-bacterial associations with high N_2-fixing capability. N_2-fixing bacteria were isolated.

Sloger, van Berkum (Maryland) isolated several kinds of N_2-fixing bacteria from crushed surface-sterilized roots of Spartina alterniflora.

2. Objective B (establishment and function of associations - laboratory studies)

Klucas (Nebraska) reported that acetylene reduction by 'Park' Kentucky bluegrass - turface soil-less model system was root-associated, greatest at 1-4 cm depth, enhanced by washing excised roots, and inhibited by surface sterilization of excised roots. Klebsiella pneumoniae was isolated from roots.

Zuberer (Texas) and co-workers indicated that moisture and combined N limit acetylene reduction in carbon amended soils under aerobic and anaerobic conditions. Inoculation of soils with Enterobacter (24/2) did not increase acetylene reduction.

Dunigan (Louisiana) found that maximum diurnal variation of nitrogenase activity by 'Saturn' rice concided with peak seasonal nitrogenase activity. Application of 100 lbs urea-N/A to 'Labelle' rice strongly inhibited seasonal and diurnal variation of nitrogenase activity.

Hubbell and Gaskins (Florida) studied colonization of grass root surfaces by A. brasilense. Inoculated roots of pearl millet and guinea grass produced more mucigel, root hairs, and lateral roots than controls. Grass roots selectively bind bacteria to descrete regions of the root. Bacteria did not bind to roots when plants received N fertilizer. Pectin lyase and endopoly-galacturonase activities were detected in cultures of A. brasilense sp 7.

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Hubbell, Vasil, and Zuberer (Florida) demonstrated that viable long-term N_2-fixing associations of sugarcane callus tissue and Azospirillum can be established. Bacteria grow on the suface and in intercellular spaces of the callus tissue. The inoculated callus did not grow any better than the control.

Sloger and van Berkum (Maryland) demonstrated that root-associated nitrogenase activity in rice is influenced by light intensity, root temperature, water stress, oxygen supply, pH, N fertilizer, and plant age. The rate of nitrogenase activity by rice roots is linked to the physiology of the plant.

Objective C (establishment and function of associations, greenhouse, and field studies)

Klucas (Nebraska) reported that 'Park' Kentucky bluegrass, grown in turface and inoculated with Klebsiella pneumoniae (W6), support acetylene reduction (195 nmol h^{-1} pot^{-1}). Turfs of 'Park' reestablished in the greenhouse and inoculated with W6 supported acetylene reduction after calculated lag periods of less than 1 hr.

Zuberer and co-workers (Texas) inoculated several grasses with native bacteria and found no correlation between dry matter production and acetylene reduction.

Dunigan (Louisana) inoculated soil with Azospirillum brazilense sp 13T and sp 13J tan, Enterobacter cloacea and Azotobacter vinelandii and found no effect on nitrogenase activity, yield of 'Saturn' rice and yield of 'Labelle' rice (fertilized with 100 Kg N/ha).

Shank, Smith, and Quesenberry (Florida) evaluated factors affecting actylene reduction (AR) activity of soil-plant cores, after overnight incubation, from forage grasses in 9 fields on 3 different soil types. AR activity increased with plant age when soil pH changed from 5.7 to 7.2 and increasing soil moisture. Low application of N fertilzer (20-30 kg/ha) increased AR activity, but larger amounts (above 80 kg/ha) decreased activity. AR activities were highest in corn, intermediate in pearl millet and lowest in guineagrass.

USEFULNESS OF FINDINGS:

Progress of studies on the occurrence, establishment, and functioning of associative N_2-fixing grass-bacteria systems conducted in both laboratory and field situations will permit a realistic evaluation of the potential of these systems for agronomic exploitation.

WORK PLANNED FOR NEXT YEAR:

No major changes in direction or emphasis in the research plans, as indicated in the project proposal, are anticipated.

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