ARDA

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

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BATCH 76 ARDA

1. SUBJECT CLASSIFICATION
A. PRIMARY: Food production and nutrition — AF00-1400-G650
B. SECONDARY: Plant production--Leguminous grains and vegetables--Tropics

2. TITLE AND SUBTITLE
Proceedings

3. AUTHOR(S)
(101) Vincent,J.M.; Whitney,A.S.; Bose,J.; (101) Hawaii Univ. Dept.of Agronomy and Soil Science; Workshop on Exploiting the Legume-Rhizobium Symbiosis in Tropical Agr., Kahului,Maui,Hawaii,1976

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1977	481p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Hawaii

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)

9. ABSTRACT This document is a record of the presentations and discussion sessions of a workshop sponsored by the University of Hawaii project to promote Nitrogen Fixation by Tropical Agricultural Legumes (NIFTAL). This workshop is a part of the overall NIFTAL project which includes a range of research, training and service programs designed to assist Rhizobium and legume workers throughout the tropics. Since legume yields are limited by numerous factors, many of which affect symbiosis, this workshop was organized to examine, in an interdisciplinary setting, a number of the major factors affecting symbiosis. The papers presented review the present situation, including potential and restraints, with respect to such topics as the role of legumes in cropping systems, soil and nutritional factors, and Rhizobium inoculation and inoculant production. Legume breeding was also a major consideration. Participants were asked to consider research and training needs in several areas including major factors limiting progress, short term vs. long term priorities, and roles of developing countries, international centers and developed countries. While the fertilizer shortage has intensified the need for research on tropical legumes and other types of bacterial nitrogen fixation in crops and soils, the need has predated this crisis. Biological nitrogen fixation (BNF) gives the LDC farmer equal access to a natural resource, nitrogen gas. The NIFTAL project is an important endeavor in broadening the total work into an international network to fully examine the various potentials of the wide variety of legumes which can be produced in the tropics for the desperately needed protein content of human and livestock diets which tend to be protein-deficient in the tropics. The full utilization of biological nitrogen fixation for both soil fertility and protein production is of crucial importance to small farmers who are unable to pay the rising costs of fertilizer.

10. CONTROL NUMBER
PN-AAF-133

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Legume crops
Meetings
Nitrogen fixation
Soil science
Tropics

13. PROJECT NUMBER

14. CONTRACT NUMBER
AID/ta-C-1207 Res.

15. TYPE OF DOCUMENT

AID 590-1 (6-74)

EXPLOITING THE LEGUME-*RHIZOBIUM* SYMBIOSIS

IN

TROPICAL AGRICULTURE

Proceedings of a workshop held at Kahului, Maui, Hawaii

August 23-28, 1976

Under the auspices of the University of Hawaii NifTAL Project
with financial assistance from the
United States Agency for International Development

Edited by

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College of Tropical Agriculture Miscellaneous Publication 145
Department of Agronomy and Soil Science
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1977

PREFACE

These proceedings record the presentations and discussion sessions of a workshop sponsored by the University of Hawaii project to promote Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL). The project is administered by the Department of Agronomy and Soil Science, College of Tropical Agriculture, and is funded in part by the U. S. Agency for International Development. The present workshop is a part of the overall NifTAL Project, which includes a range of research, training and service programs designed to assist *Rhizobium* and legume workers throughout the tropics.

Initial planning for the workshop was conducted by a committee comprised of the following:

L. R. Frederick, USAID Technical Assistance Bureau

C. N. Hittle, Intsoy, University of Illinois

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D. N. Munns, NifTAL, University of California - Davis

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A. S. Whitney, University of Hawaii NifTAL Project, *Chairman*.

Subsequent arrangements were finalized by Drs. A. S. Whitney and B. L. Koch with the assistance of the NifTAL staff.

The preparation of the proceedings was expedited by numerous individuals, and their assistance is gratefully acknowledged. Dr. D. N. Munns read and edited several of the manuscripts. Typing for production was performed by Ms. Itiuna Vaiaoga Eriksen. The various figures and tables were reduced for publication by the journalism department, directed by Mr. Jake Swan, and the media center, directed by Mr. Laf Young, of Maui Community College.

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FOREWORD

Increased exploitation of the legume-*Rhizobium* symbiosis in tropical agriculture requires the active cooperation of workers in many different disciplines since legume yields are limited by numerous factors, many of which affect symbiosis, either directly or indirectly.

This workshop was organized with the view of examining, in an interdisciplinary setting, a number of the major factors affecting symbiosis. The response was most encouraging as knowledgeable experts in various disciplines gathered to share their experience as it related to symbiotic nitrogen fixation in the tropical agriculture.

The papers presented review the present situation, including potential and restraints, with respect to such topics of rolls of legumes in cropping systems, soil and nutritional factors, and *Rhizobium* inoculation and inoculant production. In all but the last topic, legume breeding was also a major consideration. While it was not possible to fully explore all the relevant factors, the papers do provide an unusually broad treatment of the subject which we trust will prove helpful to all who are concerned with increasing the production of leguminous crops in the tropics.

Workshop participants were also asked to consider research and training needs in several areas, giving special consideration to: a) major factors limiting progress (e.g. plant, *Rhizobium*, personnel, communications, economics, social, etc.); b) short term vs. long term priorities, and c) roles of developing countries, international centers and developed countries. Edited summaries of the discussions on these matters are included in the proceedings as they provide helpful starting points for developing research and training priorities, collaborative programs, etc.

We wish to express our thanks to all of the workshop participants, many of whom invested a great deal of effort in preparing their excellent review papers and/or assisted as discussion leaders. We would like to especially acknowledge the work of Emeritus Professor James M. Vincent who served as recorder for the discussion sessions and as senior editor of the proceedings. We also acknowledge with thanks the editorial help of Dr. Donald N. Munns and the valuable assistance of Mr. John Bose in the preparation of the proceedings for publication. Lastly, we thank the wives of the NifTAL staff and Mrs. Blanche Murakami for the excellent leguminous lunches which added so much to the workshop.

A. S. Whitney

OPENING REMARKS

Donald L. Plucknett
Chief, Soil and Water Management Division
Office of Agriculture
Technical Assistance Bureau
Agency for International Development

AID considers this workshop to be very important. It is perhaps one of the first of its kind to be held, in that a wide array of disciplines and talents are represented. Soil scientists, agronomists, plant breeders, microbiologists and plant physiologists have all come together to review what is known about nitrogen fixation in tropical legumes and to discuss future potential and research strategy.

In helping to serve the needs of small farmers in developing countries, many foreign assistance agencies and national governments have struggled to find ways to help the small farmer without making his situation even more precarious because of his limited access to capital and other resources needed in production. Biological nitrogen fixation (BNF) is one of the only areas I know of where the LDC farmer has an equal access to one natural resource, namely nitrogen gas, that any other farmer does. It is not often understood that there is as much nitrogen in the air above an LDC farmer's field as there is above an Illinois soybean field. All that is required is an effective means of harvesting the nitrogen gas from the atmosphere and making it available to the crops or soil below. Hence, nitrogen fixation is one of the most equitable technical alternatives available to the small farmer.

In 1974 AID became interested in nitrogen fixation, to the point where it was willing to make an investment in research and development work in the field. A group of senior technical persons from several disciplines, including some of the senior U. S. soil microbiologists, helped us in setting priorities. Perhaps it would be useful to lay out the major features of the AID program.

The program is designed to focus on an action-oriented, problem focused research program which has the primary aim of making biological nitrogen fixation effective and available for the small farmer in LDCs. Essentially it consists of five program elements which, it is hoped, will be mutually supporting. These are: 1) a world *Rhizobium* culture and collection center at ARS-USDA, Beltsville, Maryland; 2) the NifTAL project of the University of Hawaii which focuses on identifying superior *Rhizobium* strains, matching them to legumes, and then in working out effective management systems for legumes in tropical agriculture; 3) expanded 211(d) grants in biological nitrogen fixation to some members (Cornell, Hawaii, North Carolina State and Puerto Roco universities) of the Consortium for Soils of the Tropics (COST) in order to allow them to expand their technical competence in BNF and to review the state-of-the-art in selected topics; 4) a grant program in BNF through the Cooperative State Research Service (CSRS). This program is entitled

"Limiting Factors in BNF" and is designed to draw upon the talents of individual scientists in other institutions who can help to make BNF more effective through focused research on key limiting problems; and, 5) a modest program of support for research on non-symbiotic nitrogen fixation in tropical grasses. This contract is with the University of Florida.

In addition to the five program elements above, AID has provided modest support to the National Academy of Sciences for BNF-related work such as: 1) a cooperative program on BNF in tropical grasses between NAS and Dr. Johanna Dobereiner's research group in Brazil; and 2) special NAS studies on selected legumes such as the already published "The Winged Bean a High-protein Crop for the Tropics" and two new studies, "Under-exploited Tropical Legumes" and "Leucaena: New Forage and Tree Crop for the Tropics".

This workshop, and the published proceedings to follow, will, I am sure, contribute significantly to our ultimate goal of assisting the small farmer in developing countries to increase his production of high-protein foodstuffs.

Dean William R. Furtick
College of Tropical Agriculture
University of Hawaii

It is a great pleasure to welcome the participants from research institutions in many countries to this conference sponsored by the NifTAL project of the University of Hawaii College of Tropical Agriculture. This project, which is funded by USAID, is dealing with a topic very important in world affairs. It has been my privilege to visit many of your countries. Prior to assuming my position here in Hawaii, I had an excellent opportunity while working with the United Nations to travel to many areas and become familiar with your problems. The subject of nitrogen fixation by tropical legumes has assumed major international importance due to recent world events. You are all familiar with the impact of the rapid increase in world petroleum prices and the consequent high prices of nitrogen fertilizer. This was made even more serious by the long-time cyclic trends of the international fertilizer industry in which over-investment has periodically led to oversupply followed by low prices that were unprofitable to the industry which resulted in under-investment that created shortages that led to higher prices and consequent new investment. Unfortunately, the petroleum crisis with high prices for the intermediates for fertilizer manufacture coincided with one of the periods of shortage caused by under-investment due to previous low prices. This sequence of events compounded the normal situation and led to very severe price increases and short supplies. These kinds of actions on the international scene have always resulted in sudden shifts in trends for research and development activities. The consequence of these events was to focus attention on the need for fully exploring other natural sources of nitrogen availability for crop production.

Although the focus on need for intensifying research on tropical legumes and other types of bacterial nitrogen fixation in crops and soils was the consequence of the fertilizer shortage, the need for this type of research in the tropics long predates the short-term impacts of the fertilizer cycle. The developing countries have many characteristics in common with Hawaii and our friends in Australia and New Zealand when it relates to the availability of plant nutrient nitrogen. We generally have not had an indigenous nitrogen fertilizer industry nor the potential of supporting this type of manufacture. We also are disadvantaged by the long freight haul distances from the sources of manufacture which increase cost and make our economies vulnerable to shipping strikes and other means of disruption in the supply system. These all lead to higher cost for fertilizer and problems of constant supply under all conditions. This occurs in areas where the climatic and soil factors intensify the transitory nature of the nitrogen availability in the soil and result in high nitrogen demand. These factors led many years ago to the workers in Australia and New Zealand focusing on legumes as a ready-made source for correcting these problems in their economy. The NifTAL project is an important endeavor in broadening the total work into an international network to fully examine the various potentials of the wide variety of legumes which can be produced in the tropics for their nitrogen content in the soil system and to be utilized for the desperately needed protein content of human and livestock diets which tend to be protein-deficient in the tropics.

I hope the bringing together of such a wide diversity of scientists dealing with this problem both from its disciplinary diversity and geographic diversity will prove very productive both to each of you, individually, and to the building of a better research network for solving the problem which is vital to the world food needs and particularly to the tropical areas. In the developing countries, which are well represented in this meeting, the preponderance of the agricultural community are small farmers who do not have the resources readily available to pay the price for various inputs of fertilizer. For them, the full utilization of biological nitrogen fixation for both soil fertility and protein production is of crucial importance.

My best wishes to your conference, which is aimed at addressing these kinds of problems.

RESEARCH NEEDS IN PROTEIN RESOURCES

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INTRODUCTION

The Massachusetts Institute of Technology (MIT) has prepared, for the U.S. National Science Foundation (NSF) section on Research Applied to National Needs, a detailed examination of U.S. protein resources, including conventional and unconventional types. Based on this analysis, recommendations and priorities have been proposed to NSF for expanded research support in this field. The study and its recommendations also take into account common problems in proteins -- such as nutritional quality, toxicology and processing.

It is clearly unnecessary to review here the dramatic developments during the past four years which have sharply intensified interest in research aimed at increasing the world food supply. As a consequence of these events, questions have been raised about the longer-term capability of U.S. food and protein production capacities to fill domestic needs, sustain major export demands, and still respond to urgent international emergency feeding programs.

In this context, the NSF found itself receiving, primarily from U.S. universities, requests to finance projects for research in an array of unconventional and novel protein sources. It quickly became evident that there was a lack of information which could permit adequate critical judgment of the relative priorities for such funding requests. It was clear, furthermore, that in the forseeable future the bulk of U.S. and world food supplies would have to come from traditional agricultural sources. Logically, therefore, fundamental and applied research for improving the productivity of conventional agriculture would have to receive the greatest emphasis, and priorities assigned to unconventional proteins would need to be judged in this context.

Late in 1974, NSF proposed to the Department of Nutrition and Food Science at MIT a study to analyze, in some detail, the status and potential of all relevant protein resources, and to provide the recommendations for carefully selected projects worthy of research support. This study has recognized that many aspects of such research are already receiving attention, principally from the U.S. Department of Agriculture, from the state universities and Land Grant colleges with their associated experiment stations, and from industry. Nevertheless, we believe that important areas of research vital to strengthening U.S. food and protein

productivity are either inadequately funded, or are entirely neglected, in terms of available research support. Also, in the decades ahead, there is likely to be a need to supplement conventional agriculture, and some resources should be directed toward research on possible unconventional protein sources, as well as innovative areas of agricultural research not now receiving adequate attention and support. These are the primary areas which this study has delineated and recommended for NSF research emphasis.

ORGANIZATION OF THE STUDY

Various groups of specialists, organized into committees or working groups, developed the information needed to evaluate problems affecting protein production, and the status of various resources and their research needs. Problems which were analyzed as affecting proteins generally included nutritional quality and its assessment, toxicology and food safety, processing technology, energy constraints, plant genetic potentials, nitrogen fixation, marketing of protein foods, and legal, regulatory, and political constraints which may influence introduction of novel proteins. Protein resource areas for which status analyses and research recommendations were prepared included grain crops; cereal proteins; oilseeds; food legumes; livestock production; dairy products; meat, poultry and eggs; aquatic proteins; potatoes; nonphotosynthetic single-cell protein (SCP); photosynthetic SCP; leaf proteins; and chemical synthesis of nutrients.

INSIGHTS DETERMINING RESEARCH RECOMMENDATIONS

In addition to specific information regarding various protein resources and their research needs, a number of more general conclusions emerged from the study which were helpful in formulating the research proposals. To quickly indicate some of them:

1. In the foreseeable future, U.S. agriculture will continue to supply in abundance the protein foods and feeds to which this country has become accustomed, but it will obviously do so with rising costs and increasing pressures on our land, energy and environmental resources.
2. Appropriate research and technological development must be pursued more vigorously to achieve increases in livestock and aquatic resources, and in productivity, protein quality and protein quantity of primary food crops, to meet both domestic demand and export opportunities.
3. A strengthening of U.S. food and agricultural research capabilities will be necessary to ensure continued growth of food export, a major contributor to the maintenance of favorable U.S. trade balances. If research is adequately supported, and other

appropriate policies are adopted, U.S. food export capacity at least until 1985 should increase at a rate following the trend established prior to 1972.

4. The food and feed demands of affluent industrial countries will continue to increase and will exert growing pressure on food resources available in international markets.
5. Even with probable improvements, the agricultural capacities of some major food-deficit developing countries will not soon be adequate to feed their growing populations. These countries will need to import food for many years to come, and the U.S. will play a leading role in supplying these needs, largely on regular commercial terms.
6. When food-deficit countries improve their own agricultural methods and food production, not only will the nutritional status of their populations be elevated, but they will also tend to increase their commercial food purchases in the U.S.
7. In future, the relative cost of proteins(e.g., meat and soybeans) for human and animal feeding is likely to increase more rapidly than the cost of staple sources of food or feed energy (e.g., corn and wheat).
8. It is anticipated that domestic and world demand for U.S. soybeans as a primary protein resource-- notwithstanding increasing production in other countries, such as Brazil--will grow faster than demand for other food or feed crops. While a major breakthrough in soybean (and other legume) yields may occur, it is not likely to take effect within the next ten years. Therefore, a stronger increase in soybean prices relative to those for other grain crops is anticipated. This will stimulate interest in alternative protein sources of both conventional and unconventional types.
9. It is likely that new and more productive grain crops will be developed, particularly from intergeneric crossing of cereals. Triticale is a prototype of these new crops.
10. The marketplace must reflect the increased value of crops with improved protein quality and quantity if farmers are to be persuaded to produce them. Incentives may also be needed to encourage commercial initiatives by the seed industry and others, so as to effect more rapid development of improved food crop varieties.
11. The increasing costs of agricultural food production may eventually encourage the use of unconventional protein production techniques, which are not now commercially competitive.
12. The reclamation of organic wastes of all kinds, for food and feed use, is a major priority.
13. The development and application of innovative food processing techniques, and of new science and technology, to the production of protein foods will require fair and objective regulatory standards and procedures on the part of the Food and Drug Administration, the U.S. Department of Agriculture, and the Environmental Protection Agency.

14. Any significant acceptance by the U.S. consumer of novel protein foods will require innovative marketing and promotional approaches which, to be effective, must go beyond emphasis on nutritional benefits.
15. Adoption and enforcement by many countries, including the U.S., of a 200-mile offshore fishing limit may produce even greater demand and upward price pressure for all protein supplies in international markets.

COMMON PROBLEMS AND ISSUES REQUIRING RESEARCH

Nutrition

Adequate assessment of the protein problem is handicapped by lack of knowledge in two fundamental areas: human requirements for protein at different ages and physiological states, and the evaluation of protein quality of foods. These issues are further complicated by increasing evidence that both are greatly affected by caloric excess as well as deficit.

It is apparent that the present FAO/WHO recommendations for protein requirements and, similarly, those of the National Academy of Sciences/ National Research Council's Food and Nutrition Board do not provide an adequate basis for determination of a safe practical allowance for either individuals or populations, especially under adverse environmental circumstances.

There is an urgent need to develop and apply improved methods of protein quality evaluation which are sufficiently rapid and inexpensive to be used by the food industry, by regulatory agencies, and by plant breeders seeking to develop new crop varieties of high nutritional value. These methods should be feasible with the extremely small samples likely to be available. For novel protein sources in general, and in particular for novel or conventional sources subject to extensive processing, evaluation of protein quality is especially important.

Toxicology

Toxic factors, which even in small amounts may pose a threat to animals and humans, occur in many widely used foods unless the foods are specially processed, or restricted in use. A group of inihibitors of protein digesting enzymes--e.g. the hemagglutinins (lectins) and trypsin inhibitors, which are themselves protein in nature--occur widely in food legumes (peas, beans) and oilseeds (soybeans). Fortunately, most of these inhibitors are deactivated by heat during processing. Cyanogens occur in significant quantities in foods as diverse as lima beans, almonds, cassava, and sorghum. Saponins occur in over 400 species of the plant kingdom--including sugar beets, spinach, and

asparagus. Gossypol is a toxic pigment found in cottonseed. Favism is a genetically linked disease caused by ingestion of a toxin in broad beans (*Vicia faba*). Lathyrism can be severely debilitating and even fatal in humans ingesting the legumes of the *Lathyrus* (sweet pea) species. Goitrogens, usually in the form of thioglucosides, are present in the *Brassica* species (cabbage, turnips, and mustard). A variety of anti-vitamins also occur in plant foods.

In addition to natural food toxins, substances which are potentially hazardous to man and animals may be added to foods as preservatives or coloring and flavoring agents. Some toxicants may be introduced unintentionally through the use of pesticides or fungicides. Food-safety problems associated with incomplete removal of mycotoxins and other microbial toxins are well-recognized.

In many cases, studies on experimental animals alone are not sufficient to eliminate all possibilities of adverse reactions in man, since symptoms which may appear with humans may not arise in experimental animal tests.

The need for toxicity studies is particularly evident with such proposed new protein sources as single-cell protein from both photosynthetic and nonphotosynthetic organisms, forage and leaf protein concentrates, and new legume and oilseed sources.

Innovative Technology for Protein Utilization

Traditional techniques to isolate proteins for processing into food are based mainly on extraction of a primary product, e.g. oil from oilseeds, with little concern for the integrity of the protein. As a result, the by-product proteins may become unsuitable for many useful functional applications. New technology should be developed for more effective retention of desired properties of the protein.

The basic properties of proteins from various sources require study in order to relate their molecular and physical properties, and their physical properties to their functional (or performance) properties. The critical molecular properties needing identification include molecular weights and distribution, ionization properties, reactive side chains, and primary, secondary, tertiary, and quaternary structure and morphology. Physical aspects to be characterized include hydration properties such as solubility in various media, suspendability, swelling, gelling, and rheological properties such as viscosity or apparent viscosity, yield stress, time dependency, shear dependency, and creep and relaxation. Thermal aspects include gelation and coagulation, while surface properties include hydrophilic and lipophilic properties, and surface absorption and adsorption.

For data on performance characteristics, studies are required on reactivity between starch, fat, flavoring, and other food ingredients, and on relationships between performance properties and chemical and physical properties.

Examples of process research would be concentration and isolation

of protein materials, and methodologies for restructuring protein materials, or combining proteins with other materials. The effect of processing conditions on physical properties, and the infuence of changes in the latter on organoleptic (taste, smell) characteristics of the final product both need to be studied.

Potentials for Improving Protein Quality in Plants by Genetic Means

Genticists and plant breeders have, in recent years, succeeded in developing or identifying mutations in corn, barley and sorghum which have significantly higher relative levels of lysine, as well as favorable alterations in levels of other amino acids, in their seed proteins. This achievement raises questions of the extent to which similar mutations are possible in other grains or crop plants, and also brings up the question of limits to this kind of genetic manipulation.

The single gene mutations which substantially increase lysine content in corn, barley, and sorghum involve changes in the proportions of the few protein species that normally constitute the storage protein complement of seeds, but apparently without affecting their amino acid composition.

Apparently the situation is different in the case of rice and wheat. Rice has a low proportion of prolamine to begin with, and no prolamine-depressing mutation would have much effect in altering amino composition. Wheat does contain a sizable proportion of its total seed protein as prolamine, but mutants which cause its repression are recessive. The hexaploid nature of the wheat species combines genomes of three closely related diploid species. The probability that a similar mutation would occur in all the others at the same time is obviously extremely small.

The suppression of the synthesis of less desirable protein fractions, and the secondary increase in synthesis of fractions with greater biological value, may also be effective in legumes which, in the case of *Phaseolus*, show similar storage protein heterogeneity.

Biological Nitrogen Fixation

Enhancement of biological nitrogen fixation requires greater understanding of the processes themselves, and of the organisims which effect the processes. The protein nature of the nitrogenase complex that catalyzes nitrogen fixation in organisms is fairly well understood. Research is needed to determine the crystalline enzyme components, the role of adenosine triphosphate (ATP) in the system, the mechanism whereby iron and molybdenum are incorporated into the enzyme, and the energetics and kinetics of the overall reaction.

The genes coding for nitrogenase in bacteria have been successfully

transferred from one species to another. Thus, the genetic potential exists for extending nitrogen-fixing capability to a variety of economically important organisms.

In nitrogen-fixing legumes, a better understanding is needed of the genetics of both the host species and the rhizobia. In the breeding of improved legume cultivars, insufficient attention has been given to these host-endophyte relationships. Useful application of biological nitrogen fixation to grasses requires better understanding of the genetic and biochemical compatibility of both symbionts.

Limitations to increased yield in legumes seem to be related not only to conditions affecting the availability of useful *Rhizobium* strains and the adequacy of present inoculation techniques, but, in the case of soybeans at least, to an insufficiency in photosynthate energy required to maintain optimum nitrogen inputs. A broad attack is needed on the many physiological and agronomic aspects of nitrogen-fixing systems of known or potential significance.

POTENTIALS AND RESEARCH NEEDS IN SPECIFIC PROTEIN RESOURCES

Grain Crops for Food and Feed

The tremendous and relatively unexplored genetic diversity of cereal grains provides great promise for expanded food and protein production. An accelerated genetic search for increased protein quantity and quality in the major cereals requires medium- and long-term funding. These genetic questions also call for considerable research in fundamental scientific areas relating to genetics, and in this respect represent an appropriate area of NSF concern. For example, comprehensive biochemical research on the uptake and translocation of nitrogen in crop plants, as well as related seed protein metabolism in the cereal grains especially, can lead to the development of genetic or other means for increasing seed protein quantity and quality. There is a need for expanded basic studies on the biochemical aspects of plant physiology and metabolism.

Rapid, accurate procedures for screening a variety of crops for protein nutritional quality need to be developed, for use by plant breeders, to select superior lines. Such research requires reevaluation or review of bioassay procedures and their pertinence to human and livestock animal requirements.

Increasing soybean yields by breeding requires use of germ plasm from mainland Asia. Another approach to the soybean yield problem is to determine the mechanism of oxidative photorespiration in vegetative soybeans and other legumes. This mechanism limits carbon fixation and, therefore, grain yield.

Symbiotic association of nitrogen-fixing bacteria with roots of

certain grasses and corn suggest that similar associations with cereals can be identified or developed, so as to allow large savings in energy because of reduced need for nitrogen fertilizer.

Cereal Protein Technology

The normal abundance in the U.S. of low-cost grains and their underutilized by-products suggests the desirability of initiating greater scientific and technological efforts to upgrade their food use and nutritional value.

New technologies and processes need to be perfected for separating upgraded protein products from grains and their industrial by-products. These methods would include improved techniques for producing stable edible protein concentrates from wheat milling by-products, better methods for separation of wheat gluten by aqueous processes, and separation of edible protein isolates from other cereals and their by-products. Along with an improved technology for preparing protein concentrates, new food processing applications for proteins in formulated food products should also be developed.

Research is under way in these areas, but with low priority status, in the U.S. Department of Agriculture (USDA), the state institutions, and some food industry laboratories. To stimulate this important application of technology, an economic and marketing feasibility survey is needed.

Oilseed Proteins

Soybeans represent the single largest source of oilseed protein for both animal and human consumption, and most technology for the food use of oilseed proteins relates to soybeans and a spectrum of products derived from them. A number of other oilseeds, e.g. cottonseed, peanuts, sunflowers, rapeseed, and sesame, have potential for similar utilization; however, the necessary technology for production of food-grade products from these materials is not fully developed. Additional research is needed on all these oilseeds, including soybeans, to solve problems involving flavor, functionality, color, antinutritional factors, processing technology, and many other factors.

Research and development in six food processing and utilization areas will be essential if oilseed proteins are to achieve maximum acceptance in human foods. Specifically:

1. protein and nonprotein component interactions during processing must be identified;
2. fundamental physical and chemical characteristics of the proteins of oilseeds, as related to their functional properties, need to be studied;
3. screening methods must be developed for determining functional properties of oilseed proteins with respect to their utilization in food systems;

4. identification of components responsible for undesirable flavors and development of processing technology to eliminate these from food products is essential;
5. investigation should be undertaken of modification of the functionality of oilseed proteins by physical, chemical, or enzymatic means; and
6. assessment is needed of the level at which the presence of flatus-producing oligosaccharides in oilseed products assumes practical significance, and technology to minimize this problem needs to be developed.

The efforts being devoted to these problems in USDA, the state institutions, and the food industry require stimulation, and the desirability of a selective role on the part of NSF is clearly evident.

Food Legumes

Taking into account the major problems that need to be overcome in production, utilization and marketing of food legumes, the following recommendations are offered:

In food legume production, research is needed to:
1. overcome genetic-physiological barriers to higher seed and protein yield;
2. improve control of and resistance to disease and insect pests;
3. promote greater efficiency in cultural systems; and
4. reduce the deleterious effect of adverse environmental factors.

In utilization research, the most urgent priorities are to:
1. develop convenient and rapid quantitative methods to be used by breeders, for the estimation, identification, and removal of undesirable or toxic constituents in legume seeds;
2. develop more rapid and valid methods for estimating the nutritive value of legume proteins, and apply this new technology to the improvement of protein quality in commercial seed types;
3. study combinations of legumes with cereal products and other staple foods, with the objective of improving the nutritional properties and digestibility of both; and
4. develop improved, stable, processed dry legume products that may be prepared for eating quickly, i.e., with a minimum of energy (fuel) input.

Livestock Production

U.S. animal industries are capable of producing most of the fresh meat consumed in the U.S., plus some for export, until the year 2000. However, in order to do so, great advances will be necessary in the

productivity and efficiency of animal production enterprises. These advances can be achieved through increased research in (1) recycling of animal wastes, (2) further use of by-products, wastes, and other alternative feed sources, (3) improvement of animal reproduction and growth efficiency (4) greater use of forages in animal rations, (5) increased efficiency of nonprotein nitrogen utilization by animals, and (6) development, through breeding programs, of high-efficiency animals which can effectively utilize more forage rations.

In regard to these problems, research sponsored by USDA and the state universities and experiment stations is inadequate in relation to the urgency of the problems. These agencies must expand and intensify their activities accordingly, and NSF can contribute to progress by selective funding of research in animal reproduction and growth efficiency, and by supporting innovative approaches in the recycling of animal wastes.

Animal Protein from Dairy Products

The supply and utilization of milk in the U.S. dropped from 56,700 million kg in the early 1960s (296 kg per capita in 1960) to about 53,500 million kg in the early 1970s (253 kg per capita in 1973). It is estimated that by 1980, per capita dairy food consumption in the U.S. will be down to 237 kg.

It is not likely that U.S. per capita milk consumption will increase. At present the American consumer perceives no nutritional need for increased dairy consumption, although this conception overlooks the importance of milk as a calcium source. It would take increased government-financed subsidies to produce more, but it is unlikely that the government will take steps to increase production capacity as long as other countries can produce surplus milk more cheaply than the U.S. Demands, therefore, will probably be met increasingly by imports.

This situation requires that in the next 10 years, government-sponsored research on dairy products be strengthened by 30%, and industry research by 50%, over present levels. For primary dairy products and for dairy analogs, research and related efforts are needed to:

1. improve quality and convenience of the established products, on the basis of market research guidance;
2. develop new dairy products and concepts which are marketable and competitive with fruit juices and soft drinks;
3. perfect a rapid, automated cheese production and ripening process which would increase the competitiveness of cheese in both domestic and export markets;
4. develop new processing and preservation methods for milk, so as to increase its useful storage life, reduce bulk, and make milk a significant protein resource suitable for worldwide shipment and trade;
5. develop dairy analogs based on vegetable proteins;

6. conduct nutritional studies to clarify the role of nonlipid dietary components, as well as milk-fat and dietary cholesterol, with respect to coronary heart disease;
7. optimize the food technological utility and functionality of dairy proteins as replacement proteins in fabricated foods, taking into account safety as well as nutritional factors; and
8. undertake economic and marketing studies to determine both the feasibility of increasing U.S. dairy exports, and the most favorable product mix of natural and analog dairy products.

Areas suggested for research into secondary dairy products include (1) chemical modification and upgrading of whey proteins into useful new products, (2) whey protein fractionation and characterization; (3) fundamental investigation into improving sensory and organoleptic properties of whey for applications in foods, (4) clarification of galactose metabolism in humans as a first step to wider utilization of lactose, and (5) development of whey products for the world market as protein supplements to upgrade protein-deficient diets.

All the agencies concerned with dairy research--i.e., industry, USDA, and the state universities and experiment stations--have roles and responsibilities in attacking these problems more vigorously. The call for innovations in food science also suggests that NSF could usefully support selected projects in the areas indicated.

Animal Protein from Meat, Poultry and Eggs

There is great potential for expanded development, production and utilization in the meat and egg industries. The utilization of this potential will depend on research and development inputs, and on the realism of regulatory constraints in terms of risk-cost-benefit considerations. It is also apparent that energy availability and cost, environmental issues and governmental policy decisions will have far-reaching effects on the future availability of meat and eggs.

A significant expansion of the supply of animal protein for human consumption could be brought about as a result of research into use of animal tissues which are at present diverted from the market by health or palatability considerations, or by government restrictions on otherwise safe and nutritious products.

Many packinghouse raw materials, such as visceral organs, are currently processed into livestock and pet feeds. Processes similar to those used to produce fish protein concentrates have been developed for the production of edible meat protein concentrates from the various offal tissues. However, further studies are needed in this area, as well as review of regulations to permit their use in the food supply.

Technical knowledge now available to the meat industry could result in increasing the output of processed meat products by over 50%, through appropriate extension or supplementation with plant proteins.

Consumers are concerned with the possible relationship of diet to

coronary heart disease. Attention centers on cholesterol and animal fats. Because the consumption of nutritious foods may be inhibited by adverse publicity based on inadequate scientific evidence, it is imperative that well-planned, long-range studies be implemented to clarify these questions.

Rigid and frequently outdated standards of identity discourage the development of new or improved products, which could extend the quality and variety of the national protein supply. Other standards-- e.g. microbiological standards--increase cost to the consumer without demonstrating improvements in product quality or safety as a result of their enforcement. Overregulation, in short, adds to product cost, chanelling much industry research funding into matters concerned primarily with regulatory compliance.

Broad areas of research have been receiving attention by industry, USDA, and the state universities and experiment stations, but such activity requires far greater support from funding sources than has been the case. As regards relatively basic research, there are a number of areas where NSF support would help stimulate the solution of serious and longstanding problems.

Aquatic Proteins

Most of the U.S. fishery stocks traditionally used by Americans for food are either fully exploited or depleted. There are, however, species even within the present, limited economic control zone--not to mention the larger resources within the proposed 200-mile zone--which have remained largely un- or underexploited. It is estimated, for example, that between 100,000 and 500,000 MT of capelin could be landed yearly on the northwestern Atlantic coast. This fish could be offered in the form of engineered or fabricated foods which would be more familiar to the public. Squid, likewise, is most suitably utilized in textured and engineered food products.

Much more needs to be known about the biology and behavior patterns of aquatic animals, and techniques need to be developed for locating, harvesting, preserving, and processing this resource. Polyculture, which relies on the growing-together of a number of fish species of varying feeding types, presents opportunities for attaining very high yields by taking advantage of symbiotic relationships among the different species. The high yields in such systems can be maintained without competing with nonruminant farm animals for feed. Species with maximum productivity potential in warm freshwater pond polyculture systems include carp, buffalofish, tilapia, white amur, mullet, milkfish, and catfish.

Capture fisheries represent the major focus of aquatic protein resource development. Antarctic krill, for example, is the single largest natural source of animal protein on this globe, and probably 45 million metric tons of it could be harvested yearly without compromising the availability of the resource. In view of the lead taken principally by Russia, and, to a lesser extent, by Japan, in krill harvesting and

utilization, the U.S. must now decide what commitments it should undertake in the management and utilization of this resource. It seems prudent to advise allocation of funds at levels sufficient at least to undertake a technical and economic feasibility study of U.S. participation in and utilization of the krill harvest.

As regards support for research and development, the financial and political decisions will have to be made by government, since industry will not initially invest the hundreds of millions of dollars required to perfect the tools needed to produce and utilize increased tonnage of raw aquatic material. New types of suitably equipped fishing vessels will have to be designed and constructed. New methods of locating, harvesting, preserving, and processing must be developed. New toxicological and nutritional information will be necessary to develop regulations for monitoring the quality and safety of the new aquatic food products. Investigations will be needed in biology, genetics, animal nutrition, and ecology of aquatic organisms. The National Science Foundation could most appropriately provide funding for studies in oceanography, ecology, physiology, basic biology, and other background areas.

Potatoes

The potatoe has protein levels comparable to those of cereal grains; indeed, cultivars containing 17-18% protein (dry weight basis) have been identified. The crop, clearly, does not merit the status of a "poor man's" energy food.

With the introduction of new potato varieties, this crop's yields per hectare have increased nearly 300% in the past 20 years. Through intensified breeding studies, exploration of hybridization should be continued, in order to expand adaptation, and improve disease and insect resistance, nutritive value, and yield; the ideal polyploid level for optimum productivity needs to be determined.

Systematic study is needed on factors affecting productivity and nutritional quality. These factors include optimum stage of maturity, effects of fertilization and irrigation, and possible benefits of multiple cropping with early and late maturing varieties, and with other crops. (This last factor is relevant especially to potato culture in warmer environments).

Researchers need to analyze potatoes for quantity and quality of various proteins, evaluate their amino acid composition, and determine whether or not breeding can alter protein ratios, so as to improve the overall protein nutritive quality. Simple screening methods for protein quality and quantity are needed to assist breeders in analyzing large potato populations.

Much work also needs to be done on the reclaiming and upgrading of potato processing wastes, with emphasis on recovery of proteins and amino acids.

Nonphotosynthetic Single-Cell Protein

The role of single-cell protein as a supplement to the world protein supply is now well established. Research and development on SCP production have been intense for over a decade, and as a consequence there exist a number of large SCP plants in operation for animal feed production; several more are under construction. The problems being addressed today relate to second and third generation processes for SCP production for both animal feed and human food.

No single raw material or organism will provide the ultimate SCP process. Consequently, it is essential to evaluate, in parallel, several alternatives, to appreciate fully the flexibility available in the use of SCP. There is no specific recommendation of substrates, but it is clearly the overall view of the experts that alcohols and cellulose hold the greatest potential for the U.S., and therefore should receive priority consideration.

It is believed that cell yield is the single most important economic factor in the fermentation process, and that great stress should be placed, in practice, on approaching the theoretical fermentation yield. Improved processes for RNA removal would involve study of RNA levels in cells, the role and activation of endogenous ribonucleases, and their control by means of genetic and cell physiology factors.

More efficient use could be made of the proteins within the cell if economical means for their recovery were available. Selective isolation of proteins would also be a method of avoiding the RNA problem. Studies are needed of SCP engineering properties, interactions with food constituents, and use in structure-forming operations. Dewatering and drying comprise one of the most significant cost factors in the overall process. Study is needed on membrane processes, improved methods of mass transfer, and the effect of drying on functional properties.

Research on nonphotosynthetic SCP clearly needs much wider support in the U.S., in order to increase production of protein and other useful products, especially from waste. At present in the U.S., such research and development activities are confined to industry and a few universities. The growing urgency in the U.S. for waste recovery and recycling--the need to reprocess livestock animal waste and manure for refeeding is only one example--suggests a priority area for NSF funding.

Photosynthetic Single-Cell Protein

Photosynthetic single-cell protein is produced in the cells of algae which grow in suspension in the waters of shallow, illuminated ponds. These ponds contain simple salts--such as carbonates, nitrates, and phosphates--dissolved in water. Depending on the genus, the cells may be separated from the growth medium by screening, filtration, coagulation either by flotation or sedimentation, and centrifugation.

During the next decade, steps should be taken to:

1. study the relationship of algal species to algal nutritional characteristics, including protein quality, and the influence of ratios of critical substrate nutrients, including consideration of water quality factors;
2. study productivity as a function of species, predators and pathogens, nutrient mix, physical parameters such as detention period, mixing, recirculation, and waste as a source of nutrients; and
3. study processing of algae to produce various products, including decolorized and bleached algae, algal pigments, and spun algal protein; and study algal preservation by dehydration, canning, drying, freezing and freeze drying.

Leaf Protein

Green leaves are the largest producers of protein in the world, supplying protein to other plant tissues including the crop seeds which nourish humans and animals. Indeed, the protein in the 1973-1974 U.S. alfalfa crop was almost twice as much as that in the 12.5 million MT of soybean meal utilized in the U.S. that same year.

Process research is well developed for a commercial system which would be added to a conventional dehydration plant to recover whole leaf protein. When economic feasibility studies are completed by USDA, a full-scale demonstration plant will be recommended for construction at the site of a commercial dehydration plant.

Process research for an on-the-farm-type leaf protein recovery system (in connection with silage or hay production from the press cake) has been undertaken by workers at the University of Wisconsin and elsewhere. This work involves low-cost equipment development, and further research on production and quality of the silage and/or hay produced.

Research applicable to both types of systems is needed for optimization of unit operations, for higher-value utilization of the residual juice solubles fraction, and on economic analysis of the several possible variants in the systems.

Denatured white (insoluble) products are made by heat coagulation of chlorophyll-free alfalfa juice after prior removal of the green protein fraction. A great deal of further research is needed to increase yields of this white protein, to increase its purity, and to explore its utilization possibilities in various types of food.

When *Nicotiana* (tobacco) species are used as raw leaf materials, a white protein (Fraction I protein) can be obtained readily in pure crystalline form. This tasteless, pure-white product should have special value where high purity is needed. Research is needed on growing these plants on an intensive scale, on determination of the

economic value of the crystalline protein, and on the economics of its production.

It may be concluded that leafy plants have tremendous potential for supplying an important proportion of the protein requirements of both monogastric animals and man by the years 1985-2000. What is needed is a coherent, well-financed, multidisciplinary research effort in the agronomic, genetic, chemical, engineering, nutritional toxicological, microbiological, and food technological aspects of leaf protein production.

Chemical Synthesis of Nutrients

The possibility exists of filling some of humankind's protein needs by nontraditional means, particularly by chemical synthesis. Synthetic vitamins and amino acids are, of course, already in common use. While the cost of their chemical synthesis is relatively high compared to the cost of products made by fermentation, the use of mixtures made from a common intermediate might considerably reduce the cost.

Synthesis of industrial fatty acid from carbon monoxide was conducted in wartime Germany, and increased efficiency is possible. The synthesis of glycerol for human food has also been investigated, particularly by the National Aeronautics and Space Administration, as a potential energy source for a planetary base. A more advanced approach involves 1, 3-butane diol, which is metabolized with 50% greater energy output than are sugars or glycerol.

Recent studies have shown that normal paraffins can provide some metabolic energy to chickens. There is a strong possibility that an efficient and relatively high-yielding synthesis of ATP can be developed using HCN as a starting material for adenine. All such developments, however, are clearly in the area of long-range prospects.

RESEARCH RECOMMENDATIONS

The reports of the working groups identify a large number of important research areas and projects, from which only a limited number have been selected for the recommendations which follow here. Most research which is already of major ongoing concern to USDA and the state agricultural institutions in the U.S. has been excluded only because of the special purpose of this report, and not because of any judgment as to its relative importance. The principal criterion for selection was the research's applicability to (1) an area of importance which complements or underlies traditional agricultural research and which is at present inadequately supported by USDA, the state agricultural institutions, or any other source; or (2) an unconventional area not now receiving an emphasis commensurate with its potential. No meaningful further subdivision of priority rankings can be assigned to the basic and long-term research recommended because

too many uncontrollable and unpredictable factors are involved.

Fundamental Mission-Oriented Research

1. Nutrition
   a. Evaluate protein requirements of human subjects of various ages and physiological states, by using improved metabolic balance techniques.
   b. Improve existing biochemical and biological methods for the evaluation of protein quality to reflect more closely the nutritional requirements of humans and livestock, and apply these methods to new or novel protein sources as well as processed protein foods of all types. The urgent needs of plant breeders and regulatory agencies for rapid methods of protein quality evaluation should be taken into account in this research.

2. Toxicology
   Undertake basic research on the toxicological hazards associated with new protein sources, and with conventional proteins processed in new ways. This basic research should be followed, when appropriate, by clinical trials, and by efforts to remove, through processing or other means, toxic factors identified in the animal or clinical trials.

3. Biological nitrogen fixation
   Study comprehensively the subject of nitrogen fixation in crops. This study should consist of basic biochemical and genetic research on the nitrogen and carbon metabolism of plants and microorganisms, with the objective of reducing the need for synthetic nitrogen fertilizer.

Mission-Oriented Research in Specific Resource Areas

1. Grain crops for food and feed
   a. Investigate the metabolic process of oxidative photorespiration in cereals, legumes, and leguminous oilseeds, which seriously limits fixed carbon accumulation and thus reduces potential crop yields. The objective should be to identify genetic or other means to control this undesirable metabolic process.
   b. Expand research on symbiotic and nonsymbiotic nitrogen fixation in cereals, with emphasis on development of nitrogen-fixing microorganisms compatible with host cereal species.
   c. Intensify basic research to provide new sexual and asexual

methods for breeding more productive crops with higher protein potentials. Such studies would involve identification of specific genes and gene groups, use of cell culture, somatic hybridization and chemical stimulation of gene compatibility for broad sexual crosses, as well as other innovative techniques.

d. Study more extensively the uptake of nitrogen in crop plants, and the related protein metabolism of seeds, particularly in the cereal grains, in order to identify genetic and other means for increasing protein quantity and quality without seriously compromising crop productivity and other important qualities. This will require improved screening methods to assist plant breeders in the rapid identification of desirable cultivars in protein improvement breeding programs.

2. Cereal protein technology

Develop new technologies for separating, recovering, and concentrating the protein fractions of cereal grains (wheat, corn, sorghum, barley, oats, rice, triticale, rye), and processed cereal by-products. Attention should also be given to development of new processes for reconstituting or combining cereal protein fractions with other constituents to produce attractive new protein foods such as meat analogs, meat extenders, and beverages.

3. Oilseed and legume proteins

a. Study modification of the functionality of oilseed and legume proteins, by using physical, chemical, and enzymatic methods, to facilitate development of protein materials with wide versatility and acceptability in formulated foods.

b. Intensify studies of the basic cellular and subcellular structure of oilseeds and legumes, with attention to the location and form of primary constituents (proteins, oils, carbohydrates, etc.), in order to permit development of more efficient technologies for protein and oil recovery with retention of optimal functional and nutritional characteristics.

4. Livestock animal production

a. Expand research on the feeding value of various fractions of animal and vegetable wastes for different livestock species (beef, swine, and poultry), and develop technologies, such as fermentation with appropriate microorganisms, or silage treatment, to minimize toxicity and unpalatability, and to upgrade the nutritive value of waste materials.

b. Study the carry-over, into animal tissues used for human food, of possible toxic or pathogenic factors related to feeding recycled wastes of various kinds to livestock.

5. Dairy products, meat, poultry and eggs
Develop sanitary and microbiologically safe technologies for reclaiming food products from dairy by-products, defective livestock animals, muscle and organ tissues, and poultry and eggs rejected for food use and used only partially (for livestock and pet feeding) under present food laws and grading standards.

6. Aquatic proteins
   a. Investigate more thoroughly the basic problems affecting the development and economic efficiency of aquaculture. Studies should include further identification of optimum species for monoculture and polyculture systems, and examination of the problems in breeding and culturing these species, including their metabolic and nutritional requirements and diseases to which they may be susceptible. Polyculture, which can take advantage of symbiotic relationships among several different species, should be further developed to increase efficiency of feed utilization and thus reduce competition with nonruminant farm animals for feed.
   b. Undertake a comprehensive feasibility study on the harvesting and utilization, for human consumption, of antarctic krill. The study should assess integrated harvesting technology, acceptability of possible food products, use of by-products such as chitin, and potential profitability. Since most krill resources occur primarily in international waters, apparently beyond most countries' present or future jurisdictional limits, and because the U.S.S.R. now leads in the development and application of the relevant technology, NSF should develop a collaborative effort with the Soviet Union, if an appropriate agreement can be negotiated.

7. Nonphotosynthetic single-cell protein (SCP)
   a. Clarify the nature, occurrence, and mode of action of substances giving adverse reactions in human feeding, either occurring naturally in, or produced during the processing of, bacteria, yeasts, or fungi. In addition, more economical methods are needed to reduce nucleic acid content of SCP.
   b. Develop efficient methods for the selective isolation of proteins free from nucleic acids and other undesirable constituents, and for producing such proteins with desirable functional and organoleptic properties.
   c. Expand application of SCP technology to waste processing, and recovery for animal feeding, utilizing materials such as cellulosic and other crop and animal wastes, sewage, and industrial food wastes.
   d. Improve cell yields and increase the efficiency of heat and oxygen transfer in fermenters, taking into account cooling

difficulties in tropical environments, and cell yield limitations due to inadequate oxygen supply. Improve efficiency of cell harvesting or collection, and of dewatering and drying, taking into consideration effects on protein functionality and nutritional properties.

8. Photosynthetic single-cell protein
   a. Gather more information on the toxicology, food/feed safety, nutritional value, and acceptability as food of algal sources.
   b. Develop economically feasible techniques to process algae as human food, including means for decolorizing or bleaching, recovery of algal pigments, isolation or concentration of protein constituents, and application of algae to food uses; also develop means for preservation by common technologies.

9. Leaf proteins
   a. Undertake comprehensive research, technological development, and testing of an integrated on-the-farm-type system for recovery and direct feeding, to nonruminants, of leaf protein products, including evaluation of the nutritional and toxicological implications of such feed use.
   b. Intensify development of technology for recovery of leaf protein concentrates and isolates for food use. Attention should be directed toward evaluation of nutritional and food safety aspects, as well as functionality and organoleptic qualities, of concentrates and isolates used as ingredients in formulated or fabricated foods. This work should include comprehensive study of the unique properties of *Nicotiana* (tobacco) leaves, which permit easy separation and recovery of relatively pure protein, and exploration of agronomic and other factors which relate to the optimal production of tobacco for such use.

Advanced Science and Technology

1. Innovative technology for protein utilization
   Undertake research to contribute to the basic understanding of physical and chemical properties of protein molecules. Study the separation and restructuring of plant proteins and the application of chemical engineering technology to the development of edible protein.
2. Targets of opportunity
   Make available funds for innovative exploratory research yet to be identified.

YIELD POTENTIAL FOR TROPICAL LEGUMES FROM A GENETICIST'S POINT OF VIEW

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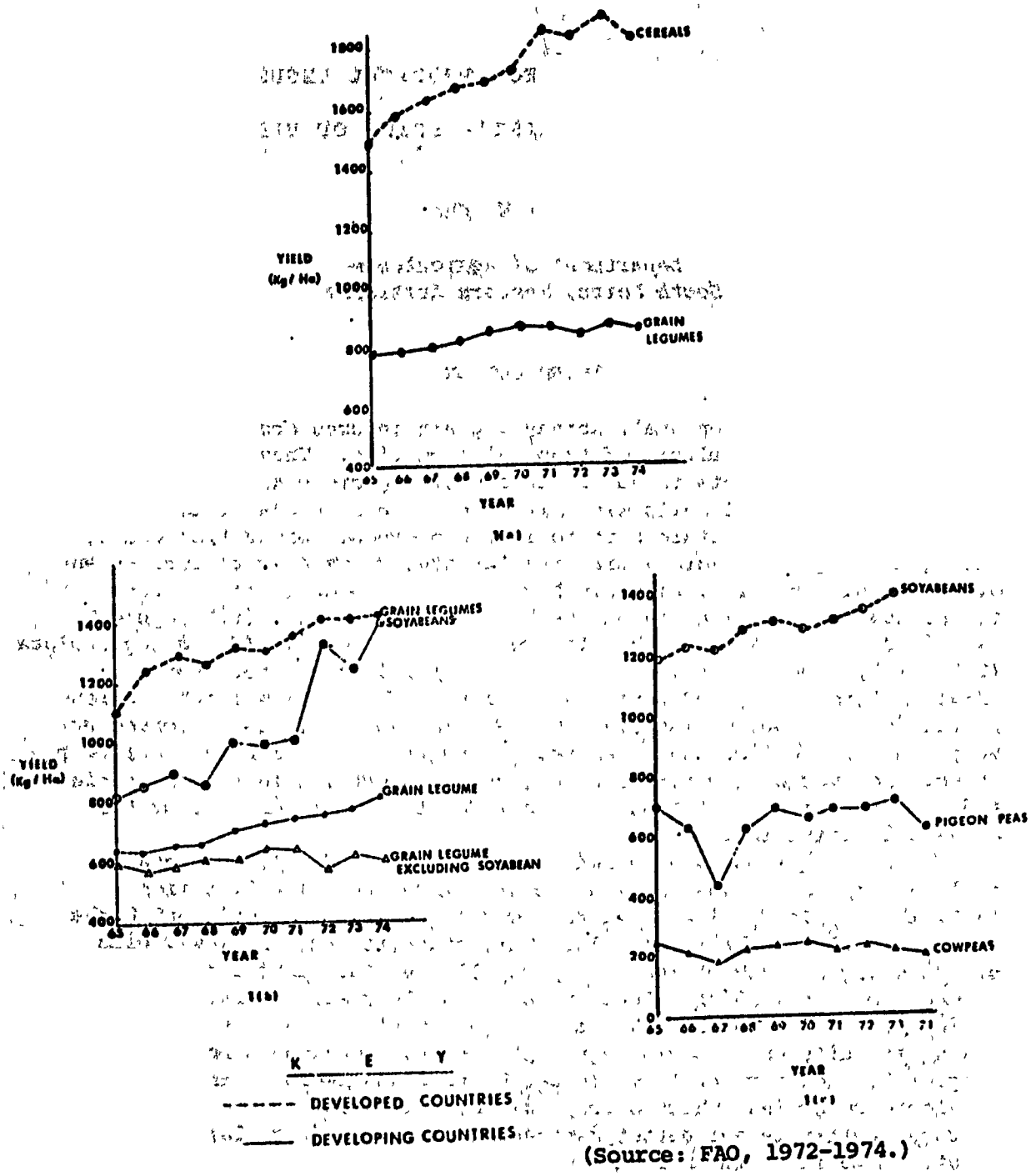
INTRODUCTION

Although grown on small acreage, grain legumes occupy an important position in the agriculture of tropical countries. They provide much needed protein in diets based on starchy staple crops and enrich impoverished tropical soils with nitrogen. There is a need to increase yield per hectare both to increase overall production and to make grain legumes a more attractive investment compared to cereals and other crops. The tropical grain legumes can be broadly placed into two categories: (i) legumes of the hot wet tropics, and (ii) legumes of mild sub-tropics and high altitude tropics. Cowpeas (*Vigna unguiculata* (L.) Walp), pigeon peas (*Cajanus cajan* (L.) Millsp.), groundnuts (*Arachis hypogae* L.) and mung beans (*Vigna radiata* (L) Wilczek.) belong to the former group whilst soybeans (*Glycine max* L.), beans (*Phaseolus vulgaris* L.) and lima beans (*Phaseolus lunatus* L.) belong to the latter group. However, wide variations in adaptation occur within each species and the area of exploitation is much wider than that conveyed by the above classification.

The grain legumes occupied a total area of 74 million hectares in 1974 compared to 734 million hectares under cereal cultivation (FAO, 1974). The yield per hectare was also less being only 559 kg/ha as against 1818 kg/ha in cereals. This suggests that the low yields of grain legumes may at least be partially responsible for their low acreage (Borlaug, 1975). An examination of 10 years statistics for yield per hectare reveals that the average yield of cereals has been rising steadily while grain legume yields have remained almost static (Fig. 1a). However, grain yield per hectare has improved in the developed countries (which occupy only a small area) due to the improved cultivars and better management practices (Fig. 1b). This point is amplified further when yields of soybeans are compared with pigeon peas and cowpeas (Fig. 1c). Because of their value in World trade soybeans have received more research effort than either cowpeas

Figure 1. Comparative grain legume and cereal yields (kg/ha), 1965-1974 decade.



(Source: FAO, 1972-1974.)

or pigeon peas. This effort has given a sustained yield improved for soybeans due to improved varieties and technology.

Most of the dramatic improvements in crop yield have originated from exceptional varieties - varieties which respond consistently to superior management. The role of geneticists in increasing the yield potential of grain legumes is therefore obvious. This paper deals with a practicing plant breeder's views on how this might be achieved. The quality aspect, apart from its relationship to yield, has been excluded from this paper. Another important aspect of grain legume growth, N_2 fixation through symbiosis, will be amply discussed in this workshop and is not dealt with here.

YIELD AND ADAPTABILITY

A comparison of the World average of yields for grain legumes in tropical areas with reported experimental yields (Table 1) indicates that considerable scope for improvement exists within currently available technology. High yield under experimental conditions may reflect both the superiority of the environment and/or the quality of genotype. Breeding of legume genotypes inherently high yielding and responsive to superior management is often complicated. For example Erskine, (1975) showed that so-called improved management of high fertility level and ample moisture in cowpeas in Papua New Guinea actually reduced the grain yield due to increased genetative growth at the expense of reproductive growth. This was associated with instability in growth habit, and may be rectified by breeding for truly determinate types. Recently world germplasm collections have been established at various international and national institutions (Rachie 1973; Vieira, 1973; Anon., 1974 a; Anon., 1971). The variation present in these collections should provide a genetic basis for substantial yield gain in the initial phases of the development in tropical countries. However as the gene pool is narrowed during selection for adaptation to local conditions, hybridization will become necessary. Grain yield often has a low heritability (Khan and Rachie, 1972; Trehan *et al*, 1970). However reports of high heritability are not unknown (Chung and Goulden, 1971; Erskine and Khan, 1976) suggesting that choice of parental material is of crucial importance.

Breeding of adaptability as well as yield is important as the tropics display a great diversity of environment, and subsistence farmers can ill afford fluctuations in yield. A great deal of attention has been focussed in the past decade on the analysis of genotype-environment interactions and on their influence on plant breeding methodology. Finlay and Wilkinson (1963) developed a regression technique for defining the stability of a genotype. The regression of a genotype's yield on the mean yield of the location estimated the stability parameter. A value of β approaching 1 coupled with higher mean yield characterized having general adaptability. Regression technique has been variously modified (Eberhart and Russell, 1966; Perkins and Jinks, 1968) and used extensively in cereals. However, regression often

accounts for only a fraction of the observed variability of the interaction term (Erskine, 1975) making the regression technique of limited application. In such situations, Baker's (1969) parameter which consists of the reciprocal of the contribution of an entry to the overall sum of squares of the genotype × environment interaction is of greater value and has been successfully applied in cowpeas by Erskine (1975). Mungomery *et al* (1974) applied the techniques of numerical taxonomy in soybeans and grouped genotypes having similar response to the environment. This technique is valuable in recognising parental material for a crossing programme designed to breed for adaptability.

There has been little reported research on the application of the adaptability concept to the breeding of grain legumes. However, the general experience in the Australian cereal breeding programmes (see Frankel, 1969) of choosing "high yielding × adapted" parental conbinations should apply to grain legume breeding as well. Such crossing programmes may require the inclusion of local material and this would lead to doubts about the value of the segregating material distributed by the International Institutes unless general adaptability is given the same status as in CIMMYT'S wheat programme.

Table 1. Yields of Major Tropical Grain Legumes

Legume	World Average (FAO 1974)		Reported High Yields
	Developed Countries	Developing Countries	(All yields are kg/ha)
Bean	792	454	3,399 (Rubaihayo *et al.*, 1975); 3,205 (Aguirre and Miranda, 1973);
Soybean	1,581	1,407	4,000 (Cooper, 1971); 3,517 (Fehr and Rodriguez, 1974);
Groundnut	1,601*	558	3,000 (Rachie, 1973); 2,240 (Laurence, 1974);
Pigeon Pea	-	531	5,000 (Anon., 1967); 2,774 (Akinola and Whiteman, 1974)
Cowpea	661	201	3,400 (Anon., 1974b); 2,800 (Rachie, 1973)
All grain legumes	901	509	

* Kernel yield calculated from "groundnut in shell" using 70 as shelling percentage.

BREEDING METHODS AND TYPE OF VARIETY

The precedent set by Johannssen in 1909 (see Allard, 1960) of pureline selection in bean has dominated the breeding of grain legumes ever since. Hybridization followed by self-pollination during selection has been commonly used with varying degrees of success. The tropical grain legumes are commonly regarded as self-pollinated crops. However, considerable variation in the extent and manipulation of out-crossing has been reported (Barrons, 1938; Rutger and Beckman, 1970; Khan, 1974). As handcrossing in legumes is laborious and time consuming, it may be advantageous to encourage natural outcrossing and utilize it for recombination. A simple breeding procedure utilizing natural out-crossing was suggested for pigeon peas by Khan (1974). Composites were constituted by mixing equal amounts of seed of 163 - 312 genotypes of roughly the same flowering time and growth habit. Composites were maintained by growing in 1000 m^2 square blocks with a beehive placed in the middle. Two pods from each plant were harvested giving enough seed to plant and similar block in the next season. The composites were utilized as dynamic reservoirs of variability yet single plant selections could be made. After 3-4 generations of random mating, composites can serve as basis for population improvement. Considerable flexibility can be effected by controlling outcrossing through spraying with insecticide and by the ability of pigeon peas to produce ratoon crops. The value of composites has, however, been seriously questioned by Hamblin and Morton (1976) who showed in beans that there is a greater chance of producing improved varieties from a large number of simple crosses than from a small number of more complex ones.

Male sterility, which has been reported in cowpea (sen and Bhowal, 1962; Rachie *et al* 1975) and soybean (Brim and Young, 1971) offers a new dimension in grain legume breeding. Brim and Stuber (1973) in soybean and Rawal (1975) in cowpea have suggested population improvement schemes which are essentially modifications of the recurrent selection method. However, their populations were to be used as base populations for single plant selection and there was no suggestion as to whether improved populations could be utilized as synthetic/multiline varieties. While the potential virtues of mixed cropping in tropical agriculture are widely recognized, little thought has been given to the problem of selecting varieties of two species which will give their best yields we when grown together. Very recently Hamblin *et al* (1976) proposed an experimental design to cater for the simultaneous evaluation of segregating populations of two species for their yield and "ecological combining ability". The procedure involves a planting plan which allows the analysis of competition (Fig. 2). Crosses are made in the two species and F_1s are raised separately. The F_2 populations from crosses within the two species are then evaluated in the above design and desirable cross combinations are located. Selection proceeds in F_3 by examining F_3 families within a cross combination using the same design. Thereafter similar selections may be used to produce "compatible" varieties for mixed cropping and/or the best line

combinations may be used as parents in succeeding cycles of selection. The above scheme deserves experimentation to verify its value in breeding for mixed cropping.

The genetic nature of existing grain legume cultivars can be summed up as follows: in advanced agricultural systems pure lines are used whereas unselected highly heterogeneous bulks are common in peasant farming conditions (Khan, 1976a). A high degree of homozygosity is however represented in both systems. The trend is towards pureline cultivars replacing heterogeneous mixtures as technology improves. This trend is often criticized on the grounds that only limited insurance against the vagaries of nature is afforded by "individual buffering" in pure lines. Multiline varieties offer scope for "population buffering" similar to that found in heterogeneous mixtures on peasant farms. Considerable research work on experimental multilines has been reported. Simmonds (1962) cited several instances where binary multilines outyielded their components for grain yield. Trenbath (1974) reviewed the biomass productivity of mixtures taking overall biomass under consideration and concluded that most mixtures are "non-transgressive" which limits their potential for maximum yield. Hamblin (personal communication) believes on theoretical grounds that if averaged over a number of seasons, multilines can exceed the average yield of the high yielding parent. While the potential of multiline varieties as high yielders can be disputed, their role in stabilizing yield in the tropical agriculture could be important and if this can be achieved, then their inability to transgress over the yield of the pureline components is immaterial. If multilines are to be used, it is necessary to investigate the effect of natural selections on the proportion of the components, unless fresh mixing at each sowing is practical. Hamblin (1975) examined the implications of seed size on survival in mixtures of beans whilst Erskine (1975) showed for cowpeas that natural selection radically altered the composition of multilines of almost uniform seed size components, both results illustrating the problems associated with introducing multilines to peasant agriculture.

SELECTION CRITERIA AND PLANT TYPE

As the heritability of seed yield is often low it has been suggested that it may be more efficient to select indirectly for yield by using more highly heritable characters that are correlated with yield. Much work has therefore been directed to correlation studies and their application to selection procedure. Adams (1967) pointed out that genetic correlations amongst yield and yield components may be due to pleiotropy or linkage or they may be developmentally induced. The developmentally induced correlations, possibly of greater value in plant breeding, may occur amongst yield components because of the competition for the limited supply of assimilates. Such correlations approach 0 under nonstress conditions and are generally negative under normal conditions which involve stress. In view of this effect

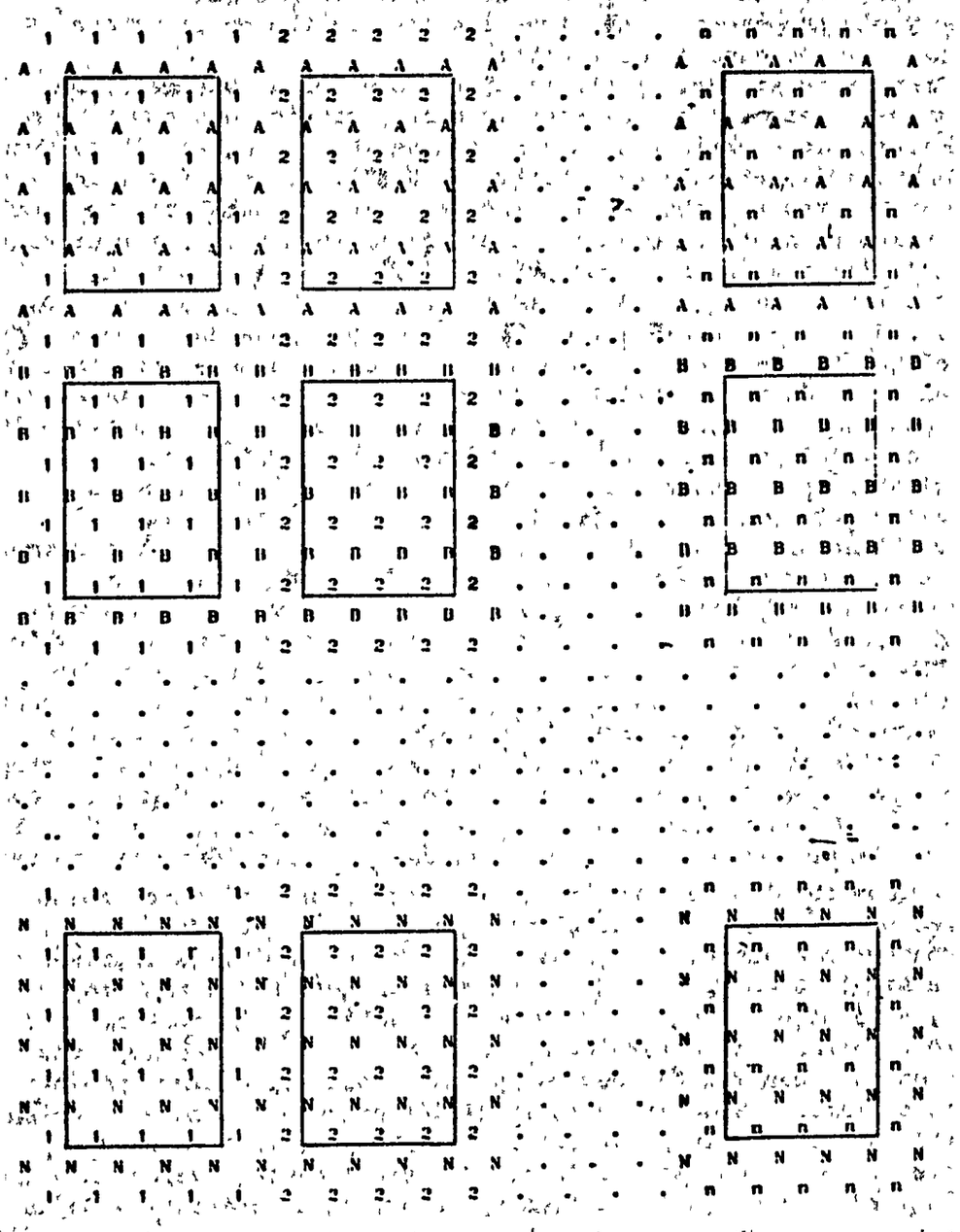


Figure 2. An example of a layout of plants for one block of an experiment with N crosses of species I and n crosses of species II, showing the test plot areas for the Nn combinations of crosses. (After Hamblin *et al*, 1976).

of environment, selections based on correlations may often lead to frustrating results. High yield will result from a balanced increase in the yield component rather than increase in the value of one component at the expense of another.

Amongst physiological criteria, two are worth consideration. In recent years varietal differences in photoperiodic response have been demonstrated in a variety of grain legumes (Huxley *et al*, 1974; Wynne and Emery, 1974; Akinola and Whiteman, 1974a). Such differences are directly related to both general and specific adaptability and should form an important consideration in all grain legume breeding programmes. Varietal differences in photosynthetic ability have also been demonstrated in various species (Egli *et al*, 1970; Ojima, 1974; Wallace *et al*, 1972; Pallas and Samish, 1974). However, whether such differences are related to the grain yield and are of value in selection is doubtful, as translocation of assimilates may be just as important. Related to the gross photosynthesis is the suggestion of Donald and Hamblin (1976) that biological yield and harvest index may be more satisfactory selection criteria due to their causal relationship with yield.

Related to selection criteria is the concept of "ideal plant type". Plant breeders generally have a mental picture of the favourite plant type based on their experience and imagination, and during early generations selection is commonly based on visual assessment. Donald (1968) formalized this concept introducing plant models which he called "ideotypes". Models are constructed from the available knowledge of the physiology of the plant and the growing environment. The ideotype thus has specific adaptation. The possibility of an ideotype for wide adaptation is not discounted but our knowledge of crop physiology is too limiting at present to contemplate such models even in theory. Amongst grain legumes, beans have attracted most attention from the "plant architects". Adams (1973) proposed ideotypes suitable for various farming systems including monoculture, mixed cropping, multicropping, and slash and plant agriculture. The ideotype for monoculture was suggested as having the central axis represented with a single stem or with 2 - 4 basal branches, non-bunch growth habit, a large node number but without lodging problems, small and numerous leaves, and determinate growth habit. This ideotype was based on the need for an open canopy structure for maximum photosynthetic rates. However, Hamblin (1976) argued that under English conditions a close canopy structure is likely to lead to a better utilization of resources. This strengthens Evans' (1973) commentary on Adam's (1973) paper that the ideotype approach at present leads to specific adaptation. Although the ideotype approach may be appropriate in the quest for the highest possible yield, the concept of specific adaptation is not compatible with peasant farming situations in the tropics. Here, breeding for general adaptability by examining genotype × environment interactions offers a more practical solution.

There is a tendency amongst modern grain legume breeders to favour the determinate plant type, which is compatible with mechanical harvesting. It is emphasised that indeterminate plant type is often preferred

in peasant farming due to their ability to provide foods over longer periods of time, and should not be overlooked in a breeding programme.

DISEASE AND INSECT PEST RESISTANCE

The multiplicity of insect pests and diseases in the tropical environment dictates the highest priority to their genetic control. Varietal resistance to fungal, bacterial, viral and nematode diseases and their genetic basis has been reported in various grain legume species. Resistance is often monogenic and is governed by dominant (Brantley and Kuhn, 1970; Bernard and Cremeens, 1971; Augustine *et al*, 1972; Kilen *et al*, 1974; Thomas *et al*, 1975) or recessive genes (Provividenti and Schroeder, 1973; Rogers *et al*, 1973; Reeder *et al*, 1972; Rao and Patel, 1973). Few cases of multigenic resistance have been reported (Anon., 1970). A complex genetic mechanism involving four genes has been reported in common mosaic virus of beans where four recessive allele s of locus "a" confer resistance against one or more strains in the presence of an allele s only (Anon., 1975). Another gene L results in necrosis depending upon the combination of a and s. However a recessive gene b confers resistance to all races. Hartman (1971) reported that resistance to root knot nematode *(Meloidegyne incognita)* in beans is governed by 3-4 recessive genes of equal effect. Resistance to *Xanthomonas phaseoli* in beans was also reported to be polygenically inherited with a narrow sense heritability of 14% (Dermot and Schuster, 1974).

Insect resistance in plants though widely recognized is often left out of breeding programmes in view of the difficult screening techniques. There have been studies of the genetic nature of resistance to a variety of insect pests, including pod borers (Frey and Cuthbert, 1975), spider mite (Parameswarappa *et al*, 1974) and aphids (Sama *et al*, 1974). While it is not uncommon to find clear cut genetic mechanisms of resistance as in spider mite resistance in soybean (Parameswarappa *et al*, 1974), insect resistance often displays complex inheritance with low heritabilities (Frey & Cutbert, 1975).

There has been a great deal of discussion centred around the genetic nature of resistance that gives permanent and/or prolonged protection. Caldwell (1968) defined two types of resistance - specific resistance against a pathogenic race and general resistance against all races. Commonly the former type of resistance is governed by a single or few genes whereas the latter type involves multigenic action. However this is not a hard and fast rule and several instances of single genes giving general and prolonged resistance are known (see Caldwell, 1968). The permanence of resistance is also dependent on the nature of the pathogen. Both types of resistance have potential applications in the breeding of grain legumes, for whilst specific resistance is easily manipulated and provides protection quickly, the long term breeding aim should be general resistance. The same argument applies to insect resistance. However insect resistance offers a greater challenge to

plant breeders as often both physiological and morphological features contribute to the mechanism of protection and defence. Insect resistance should be considered when designing an ideotype; this should open a new era of cooperation between the crop physiologists and entomologists. It can be stated that the field of genetic resistance to insects and diseases in grain legumes has been somewhat neglected and its potential has been underestimated. The International Institutes of Agriculture have now started massive screening programmes involving thousands of genotypes. Their results indicate that disease and insect resistance in grain legumes is of common occurrence. The utilization of these genes in a rational programme of pests and disease control offers a great challenge.

Janzen (1973) pointed out the positive relationship between the anti-nutritional factors and insect pest resistance in the tropical environment, and Hamblin and Kent (1973) and Bohlool and Schmidt (1974) have suggested their implication in nodulation. A breeding programme against antinutritional factors must be balanced against long term effects on the control of pests and efficient nodulation.

SPECIAL TECHNIQUES IN GENETIC MANIPULATION

Amongst special breeding techniques, interspecific and intergeneric hybridization and mutation breeding have received some consideration in the past. Haploid pollen plant culture has a great potential in radically reducing the time taken in breeding new varieties. This has not been perfected in any tropical grain legume species. Although callus formation in another cultures has been found in soybeans (Ivers *et al*, 1974) and winged bean (T. Khan, unpublished), differentiation has not been observed. In soybeans, it was confirmed that the callus was of diploid tissue. A breakthrough in this technique in the near future will be of great assistance to the grain legume breeders.

A number of reports on interspecific and intergeneric hybridization in tropical grain legumes are available (Smartt, 1970; Varisai, 1973; Baker *et al*, 1975; Evans, 1975; Raman and Gopinathan, 1975). The utility of this technique lies mainly in the transfer of disease resistance from wild species, as well as in recombining the desirable features of two or more cultivated species. McComb (1975) in a critical review pointed out the irregularities in reporting of the hybridization results and discounted the general possibility of intergeneric hybridization amongst grain legume species. She advocated the use of the more recent technique of protoplast fusion (McComb, 1974) in the quest of intergeneric as well as interspecific hybrids.

The application of mutation breeding in seed protein improvement has received impetus under the leadership of the International Atomic Energy Agency (Sigurbjornsson and Luse, 1975). Mutation breeding has been successfully utilized in the production of mutants representing variations in growth habit, maturity, yield and a score of other characters (Ojomo and Chedda, 1972; Kuun *et al*, 1973; Mujib and Greig,

1973; Sreerangasamy *et al*, 1973). In the author's opinion at this stage of development, mutation breeding can play two important roles: first, in the evolution of rare characteristics such as male sterility, resistance to diseases and pests etc., and second, in the revival of little known grain legume species. Potential of such species has been recognized so late that most of the genetic variability has disappeared. Mutation breeding should help to enrich the genetic basis of these species so that viable improvement programmes can be initiated.

UTILIZATION OF UNDEREXPLOITED SPECIES

Rachie (1973) has given a comprehensive list of tropical grain legumes amongst which a number of underexploited and little known names emerge, including bambara ground nuts *(Voandzeia subterranea* (L.) Thouars), guar (*Cyamopsis tetragonoloba* (L.) Taub.), mung beans (*Vigna radiata* (L.) Wilczek) hyacinth beans (*Lablab niger* Medic), rice beans *(Phaseolus calcaratus* Roxb.), jack beans *(Canvalia* DC.), lima beans *(Phaseolus lunatus* L), winged beans *(Psophocarpus tetragonolobus* (L.) DC.) and velvet beans (*Mucuna pruriens* DC var. *utilis* Wall.). In addition Farrington (1974) has pointed out the potential of many small seeded legumes cultivated presently as forage. Amongst these, the potential of mung beans has now been recognized and a germplasm collection has been established at the University of Missouri (Yohe and Poehlman, 1972). The potential value of winged beans was recognized in 1974 and since then a limited number of scientists have put in a great deal of effort towards its development. A number of the above species have exhibited very high yield potential under experimental conditions. Some of the reported figures are 5024 kg/ha in velvet beans (Rachie, 1975), over 4000 kg/ha in jack beans (Khan, unpublished) and 4590 kg/ha in winged beans (Wong Kai Choo, 1975). The above legume species however suffer from some limitations which prohibit their large scale exploitation at present. For instance winged beans and velvet beans are both indeterminate climbers, whereas jack bean seed contains a quantitative toxin. The genetic variability available to plant breeders at present is too small to develop a successful breeding programme to improve these species, and there is an urgent need to initiate concerted efforts to collect and establish genetic reservoirs and enrich these with mutation breeding.

CONCLUSION

Genetic improvement of yield potential will be one of the most important factors in the development and promotion of the tropical grain legume species. Yield improvement in a plant breeding programme can be brought about by increasing the yield potential by the accumulation of yield genes, and (or) by removing yield barriers such as lodging, diseases etc. The evidence presented shows that improvement in both

the above aspects is possible and should be achieved simultaneously to bring about a dramatic rise in yield levels. Adaptability is of vital importance and should form an essential part of breeding programmes. Such an emphasis on yield improvement, however, comes in direct conflict with the improvement in quality as selection for yield may depress the quality and quantity of protein (Oram and Brock, 1972). A compromise of breeding for yield while keeping quality at an acceptable standard is therefore advocated as offering the best use of the limited research resources currently available.

The genetic planning of tropical grain legume improvement should attempt to avoid the mistakes of the past, and should be based on both varietal and species diversity to cater for long term stability to changing managerial environments and markets. Greater research resources should be directed to the underexploited legumes as it is amongst these species that one is likely to stumble on plants like winged beans whose green pods, peas and flowers will provide a nutritious vegetable, whose seeds will be used as high protein pulse and stock feed concentrate and in the extraction of oil and protein, whose tubers will provide a high protein delicacy for the table, and which above all will enrich the soil with nitrogen (Khan, 1976 b).

There is a need for grain legume breeders to employ a more imaginative and open minded approach. The ideotype approach, though controversial, should not be altogether ignored as it has important implications for specialized objectives such as insect resistance, suitability for technological level and local adaptation. The recombination potential through utilization of male sterility and population breeding should be of added help in this respect. The use of multilines and mixed cropping should be encouraged wherever possible and breeding programmes should be designed to produce cultivars suited to these purposes.

Last but not least is the need to conserve the genetic treasury of grain legumes. There has been a good deal of work done in recent years on recognized species. There is a need to give similar attention to the currently unimportant species and their related wild species. The present collections are more or less a monopoly of the International Institutes and steps should be taken to disseminate this variability into national programmes which are at present running on very narrow genetic bases.

ACKNOWLEDGEMENT: The help of Dr. John Hamblin who read the manuscript and offered helpful criticism, is gratefully acknowledged.

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YIELD POTENTIAL FOR TROPICAL PASTURE LEGUMES

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INTRODUCTION

Tropical pasture legumes, like their temperate counterparts, are of value because they form effective symbioses with *Rhizobium* and can grow and reproduce in soils low in nitrogen. This ability, which has often been doubted by some agronomists, is now extremely well documented (Henzell and Norris, 1962; Whitney *et al.* 1967; Date, 1973) and need not be elaborated any further in a workshop such as this. Perhaps what needs to be stressed is that the quantity of nitrogen fixed is very largely dependent upon the dry matter yield of the particular legume (Jones *et al.* 1967; Jones, 1972). This in turn is a reflection of the interaction of the legume genotype and the environmental conditions experienced during growth. Superiority in nitrogen fixation is not therefore an inherent characteristic of a given legume species since the genetic potential is usually limited by some environmental factor such as water supply, temperature, nutrient availability or defoliation pressure from grazing animals. Thus the ranking of a collection of legumes in ability to fix nitrogen will usually change when grown in different soils or in different climatic environments because these factors differentially affect the yielding ability of the legumes.

In assessing the potential of tropical pasture legumes, it is also important to note three important differences compared with the assessment of the potential of grain legumes.

Firstly, pasture legumes are not usually re-sown annually. Therefore regeneration from seed without further cultivation treatment, or perenniality of the original plants, is essential for success. This then means that the plants have to withstand much greater environmental stress than do legumes grown for grain. Secondly, pasture legumes are usually grown in association with other species - usually grasses - and their potential is expressed in association with such species under a system of cutting or grazing. Thirdly, the potential of the pasture legumes can only really be assessed in terms of animal production - the major end point of any forage programme and virtually the only saleable product from pastures.

These three differences combine to make the assessment of the potential of pasture legumes a much more prolonged and complex process than the assessment of grain legumes.

In this paper, I will briefly discuss the historic development of tropical pasture legumes, consider the biological limitations to yield potential in these legumes and review the production currently being achieved from these legumes under field conditions.

HISTORY

Development in the Wet Tropics

It was natural that the use of sown legumes should first be made in the wetter tropics where plantation agriculture was developed. In these regions, forest areas were cleared for both the plantation crops and other crops needed for the labour force. On these cleared forest areas, animals were fed on areas colonized naturally by grasses and associated species as well as on areas specifically planted with grasses. The legumes, sown originally as cover crops or for green manure, also served as feed for a few livestock and were later to be developed as pasture legumes sown specifically for livestock. Legumes used successfully for such purposes are still of major importance in the wetter tropics - namely: *Centrosema pubescens, Pueraria phaseoloides, Calopogonium muconoides and Stylosanthes guianensis* (Warmke *et al.*, 1952; Vivian, 1959; Vincente-Chandler *et al.*, 1964; Teitzel and Burt, 1976).

Development in the Sub-Tropics

The second phase of development of tropical forage legumes occurred in the sub-tropics of Australia from 1950 onwards. Under the impetus of Dr. J. Griffiths Davies, a new era in pasture development for these areas dawned. The concepts underlying this development have been clearly presented (CSIRO 1964; Shaw and Bryan, 1976) and these concepts have formed the basis for pasture development based on legumes in other parts of the world.

For these sub-tropical areas, the legumes noted above for use in wet tropical areas proved to be unsuitable. In particular, they lacked cold tolerance and had too short a growing season. Furthermore, they were not drought resistant and this further limited their use in the drier sub-tropics. The outcome of this Australian work was the development of the sub-tropical pasture legumes, Siratro, *Desmodium intortum and D. uncinatum, Lotononis bainesii* and *Trifolium semipilosum*, the forage legume *Lab-lab purpureus* and the shrub legume *Leucaena leucocephala* cv. Peru (Davies and Hutton, 1970). Also from this work the value of a naturalized legume species Townsville stylo *(S. humilis)*, in markedly improving animal production was established (Shaw 1961).

Development in the Dry Tropics

The development in the seasonally dry tropics of Australia occurred

later and was based initially on the results obtained with *S. humilis* pastures (Norman, 1959, 1968; Northern Territory Administration, 1965). Until recently this species, and another annual species *Macrotyloma uniflorus*, were the only legume species which could be used in these extensive seasonally dry tropical areas. From 1970 onwards, a range of legume species - mainly in the genus *Stylosanthes* introduced from central and south America - have shown promise for these areas. *S. hamata* cv. Verano and *S. scabra* cv. Seca, a short-lived and true perennial respectively, have been released as cultivars as a result of a comprehensive research effort (Edye *et al.*, 1973, 1975, 1976). Other *Stylosanthes* species and genera such as *Centrosema*, *Macroptilium* and *Desmanthus* contain introductions which are persistent in these environments and offer scope for the development of cultivars which are productive, palatable and persistent (Burt and Reid, 1975).

Historically, therefore, the development of tropical pasture legumes has proceeded from the favourable areas of the wet tropics where the original vegetation was often rainforest, to the more unfavourable environments of the dry tropics where the original vegetation was either grassland, shrubland or open woodland.

It is interesting to note that of the tropical legumes in commercial use in Australia in 1976, only one cultivar has resulted from a plant breeding programme - namely Siratro (*Macroptilium atropurpureum* cv. Siratro) (Hutton, 1962). The remaining legumes are either direct introductions or selections from within naturalized populations (Barnard, 1972).

LIMITATIONS TO YIELD

Genetic Limitations

Clearly the genetic constitution of any legume will set a ceiling to its yielding capacity. Exploitation of new environments in pasture development has been achieved mainly by the use of different genera and species rather than by the selection or breeding of those previously used. In some instances, genetic improvement has been possible by selection from a wide range of material obtained from different geographical areas. Thus for the wet tropics the cultivars Cook and Endeavour have given higher yields than Schofield stylo particularly in Spring and this may reflect their source of origin at altitudes above 1,000m in south and central America respectively (Barnard, 1972). Further evaluation of much wider range of *Stylosanthes guianensis* introductions indicates that except in the sub-tropics, where earlier introductions of *S. guianensis* were unproductive, these have very little yield advantage over the established cultivars (Edye *et al.*, 1976). Similarly, Edye and Cameron (1975) showed that recent introductions of *S. humilis* into Australia had no higher yielding ability than the existing cultivar Gordon.

In *Centrosema pubescens* the cultivar Belalto was selected on the

basis of cool season growth performance. During this season yields were up to three times that of the common centro, a difference which was partly attributed to relative freedom from *Cercospora* leaf spot disease and *Tetranycus* sp attack (Grof and Harding, 1970). Evaluation of twenty-four other accessions of *C. pubescens* showed little genetic variation and none was superior in yield to cv. Belalto (Clements, 1975). This was in marked contrast to the variation in vigour which occurred in *C. virginianum* (Clements, 1975), however, for this species no standard cultivar exists for comparative purposes.

Although considerable variation occurs in *Desmodium* accessions (Rotar, 1970) there seems little scope at present for increasing yields above those obtained with *D. intortum* cv. Greenleaf (Imrie, 1973 and personal communication).

Considerable increases in yield have been achieved by breeding in the species *Macroptilium atropurpureum* (Hutton, 1962) and lines currently being evaluated outyielded Siratro by up to 50 per cent (E.M. Hutton, personal communication).

The shrub legume *Leucaena leucocephala* exhibits large variation for yield among different accessions (Hutton and Bonner, 1960; Oakes and Skov, 1967; Brewbaker *et al.*, 1972) with the shrubby Hawaiian type giving low yields (to 6 t DM/ha) and the taller lines from Peru and El Salvador giving high yields (to 15 t DM/ha). Yields 30-40% above those of cv. Peru have been obtained from cv. Cunningham which was produced by breeding (Guatemala x Peru) (Hutton and Beattie, in press; unpublished results of author).

A great diversity of genetic material is clearly available in nature, both between and within legume genera. In the wet tropics and the moist sub-tropics, it appears that much of this natural variability has been exploited, however, the increasing importance of diseases in legumes, particularly of anthracnose *(Colletotrichum gloeosporoides)* in *Stylosanthes* (D. Cameron, personal communication) will necessitate the presence of genes conferring resistance if high yields are to be maintained. Fortunately, variability exists for resistance to a number of plant diseases (Sonoda *et al.*, 1975). In the drier sub-tropics and the seasonally dry tropics, a large resevoir of genetic material has yet to be collected and evaluated. Only when such collections have been studied can meaningful breeding objectives be assessed and meaningful breeding programmes implemented.

Climatic Limitations

Water:

With the exception of the very wet tropical areas, tropical legumes will encounter periods of moisture stress during the year and this will result in potential yield reduction, particularly if such moisture stress occurs in the warm months when growth is active. The stress is not only dependent on the total rainfall but on its distribution, the soil characteristics and the rooting pattern of the legume concerned. These

factors determine the amount of water that may be transpired by the crop and this in turn is often related to yield. Thus, McCown *et al.*, (1974) showed that dry matter yield of Townsville stylo based pastures was closely related to actual evapotranspiration estimated from a simple water balance model. In the unfertilized *S. humilis* - *Heteropogon contortus* pastures, P fertilized *S. humilis Cenchrus ciliaris*, and N and P fertilized *Cenchrus* pastures, the calculated dry matter production per cm of evapotranspiration was approximately 50, 100 and 200 kg DM respectively. These values may be compared with a value of 74kg legume DM for every cm of effective rainfall in the 64 to 88cm range in a Siratro - *Paspalum plicatulum* pasture in S.E. Queensland (Jones *et al.*, 1967). Unfortunately, there are no data comparing the water use efficiency of different tropical legumes growing on the same site. However, the large yield differences which occur between legumes in dry environments suggest that large differences do occur. In particular, shrubs and trees (e.g.,*Leucaena)* retain leaf for longer periods into the dry season. If such relations were known, then the prediction of potential legume yields in any given moisture stress situation could be envisaged.

In dry areas, the yield that is retained in the dry season may be as important as the yield of legume grown, for the in absence of herbage conservation the fate of the standing or fallen legume may well determine the productivity of the pasture in terms of animal output.

Temperature:

In the sub-tropics, low temperatures invariably limit the growth of tropical legumes for part of each year. Even in the tropics, low temperatures and even frosts can severely reduce the feeding value of the stand-over feed even if the dry matter yield is not severely reduced. From controlled environment studies and from field measurements the tropical legumes appear to fall into at least two groups, referred to by Sweeney and Hopkinson (1975) as the "warm tropical legumes" and the "cool tropical legumes". The latter group is characterized by better production at lower temperatures and a marked depression in growth at mean daily temperatures above about 27C. Although all the species set out below have not been tested at the full range of temperatures, this group appears to include *Desmodium intortum, D. uncinatum, Glycine wightii.* (Ludlow and Wilson, 1970; Whiteman, 1968; Sweeney and Hopkinson, 1975), *D. sandwicense* (Whiteman, 1968), *Macrotyloma axillare* (Herridge and Roughley, 1976) and possibly *Lotononis bainesii* and *Trifolium semipilosum* ('t Mannetje and Pritchard, 1974). It is perhaps not surprising to note that these species are either of sub-tropical origin or from medium altitudes in the tropics. The warm tropical species, which include *Macroptilium, Centrosema, Pueraria, Stylosanthes*, appear to have a yield plateau above 27°C (Whiteman, 1968; Sweeney and Hopkinson, 1975) and their productivity is more severely depressed at lower temperatures (Ludlow and Wilson, 1970; 't Mannetje and Pritchard, 1974; Sweeney and Hopkinson, 1975).

The production of *L. bainesii* under different temperatures and

daylengths in the study of 't Mannetje and Pritchard (1974) was outstanding in comparison with other legumes and in addition it is known to be tolerant of frosting in S.E. Queensland (Bryan, 1972). Such performance in the field is, however, rather short-lived and other factors - including competition from associated species in the presence of available nitrogen - overrides its rather remarkable ability to produce in such widely different climatic regimes. Irrespective of level of yield, for each legume there appears to be a linear decrease in growth rate over the mean temperature range 28°C to 14°C; indicating the very pronounced limitations to dry matter yield imposed by temperature in most situations except the hot wet tropics. No appreciable growth of *C. pubescens* in spring in sub-tropical Queensland occurred until minimum temperatures exceeded 13°C or maximum temperatures exceeded 25.5°C (Bowen, 1959). Respective values for Siratro were 14°C and 21°C (Jones, 1967) but for *D. uncinatum* growth occurred when minimum temperatures exceeded 10°C (Whiteman and Lulham, 1970). Field observations also indicate that frosting during the winter retards the rate of regrowth in spring with the result that associate grasses, already growing, are even more competitive. These limitations imposed by low temperatures are particularly relevant at high altitudes in the tropics where minimum temperatures are often low. For this reason, it has been suggested that for large areas of E. Africa and Ethiopia above an altitude of 1,500m and where minimum temperatures are 15°C or less, a search for adapted Mediterranean and temperate legumes may well be more rewarding than the use of tropical legumes currently available (Wheeler and Jones, in press).

At high temperatures, particularly high night temperatures, some legume species fail to flower and continue vegetative growth. For example, in New Guinea, sowing of *Lab-lab purpureus (= Dolichos lab lab)* for seed production is not recommended where minimum temperatures exceed 64°F (18°C) (Hill, 1967). At low temperatures, especially with low minimum temperatures, seed formation in *S. humilis* may be adversely affected (Skerman and Humphreys, 1973; Schoonover and Humphreys, 1974) and for this annual species any reduction in seed production could result in a corresponding loss of production of dry matter in the following season.

Radiation and daylength

In the tropics levels of radiation are usually high and are often the least variable component of the climate from year to year (Coaldrake, 1964). Although radiation is closely related to photosynthetic rate when other factors are non-limiting (Ludlow and Davis, 1975) it is doubtful if radiation levels *per se* limit the productivity of tropical legumes except where the cloud cover in the wet tropics is a persistent feature of the environment. Under these conditions in a legume-grass association it has been postulated that the legume would be relatively more favoured than when grown under high radiation levels. This is due to a greater decrease in net assimilation rate and a smaller compensatory increase in leaf area ratio for the grasses than for the tropical legumes with shading (Ludlow, *et al.*, 1974). Shading reduced

yield in a range of legumes but the effect on some legumes such as Siratro was far greater than on others (Whiteman *et al.*, 1974). However, as these authors point out, the yields of Siratro under the highest shading treatment were greater than those of the other legumes tested. Yield reduction in Calopo under shading has been associated with a decline in the number of runners produced rather than to a reduction in their individual weight (Ludlow *et al.*, 1974). It is not known if this occurs with other legumes nor whether there are differences between legumes in their ability to maintain runner production when shaded.

Daylength can influence yield by reducing the vegetative growth of species which become reproductive under certain daylengths, and also by a direct effect of daylength on yield. This latter aspect has not been generally appreciated but in controlled environment studies yield was reduced at daylengths of 11hr compared with 14hr in a range of tropical legumes and was most marked in *M. atropurpureum* and *D. intortum* ('t Mannetje and Pritchard, 1974). In the sub-tropics, daylength and temperature decrease together and so a marked decline in productivity can occur.

Perhaps the most marked effect of daylength on productivity of a tropical legume occurs with *S. humilis*. Flowering is induced only when the daylength is shorter than a critical photoperiod of from 11½ to 13 hours according to the maturity type of the particular accession (Cameron, 1967). Using a mid-season maturity type grown in Brisbane, 't Mannetje and van Bennekom (1974) produced plants with yields at flowering which varied from 0.05g to 54.75g/plant simply by varying the sowing date. In the field the unsuitability of areas near the equator with daylengths of about 12hr result in low yields of this annual species, and even at lower latitudes choice of the correct maturity type is important to achieve high yields. Even in perennial species cultivars with a prolonged vegetative phase are usually more productive. For example, Greenleaf desmodium is higher yielding than many other desmodium lines in S.E. Queensland but even higher yielding lines are possible by still later flowering material if the water regime enables growth to continue into autumn (B.C. Imrie, personal communication). A similar situation occurs with Tinaroo glycine, which, because of its short day requirement, remains vegetative long into the autumn and is capable of higher yields providing water is available to capitalize on this ability (Edye, 1967). In the long term, however, plants which rarely set seed may be at a disadvantage if regeneration from seed is a pre-requisite for success in that environment. With the wide range of material available it should be possible to provide genotypes which perform best in specific sequences of daylength and rainfall.

Soil and Nutrient Limitations

Our knowledge on the interaction between soils and legume species is very meagre. In general, tropical legumes grow well on neutral to

acid sands and loams provided mineral nutrients are not limiting. Heavy clays on the other hand, are notoriously difficult sites for legume growth and persistence. Exceptions do occur, however, and *Leucaena leucocephala* and *Clitoria ternatea* are two species which thrive on alkaline heavy clay soils (Parbery, 1967; C.G. Blunt, personal communication). The genus *Stylosanthes* is generally regarded as adapted to acid sandy soils but within the genus, and even within species of this genus, certain ecotypes are known to be adapted to alkaline clay soils, e.g., *S. hamata* (Burt and Miller, 1975).

At the C.S.I.R.O. Lansdown Research Station near Townsville there is clear evidence of the effects of soils on legume species performance. On fertilized duplex solodic soils, species such as *Leucaena leucocephala* and Siratro do not grow although both these species do extremely well on alluvial soils, a red earth, and red podzolic soils. *S. humilis* and *S. hamata* grow well on all these soil types. We may speculate that on the solodic soil the excessive wetness during the growing season and the excessive dryness in the dry season are involved in these differences but the real reasons are not known. In Brazil the superiority of Siratro compared with *Glycine wightii* on the red sandy soils is most apparent, whereas the growth of Glycine on adjacent areas of red clay-loam soils with a higher base saturation far exceeds that of Siratro (Jones, 1974a). Even within the species *G. wightii* some cultivars have much wider edaphic adaptability than others. For example Tinaroo glycine is particularly adapted to fertile soils in high yielding environments but performs poorly in less favourable conditions compared with cv. Clarence and cv. Malawi and with Greenleaf desmodium (Gartner, *et al.*, 1974). A clearer understanding of the relations between soil characteristics and legume growth and persistence would greatly improve our ability to efficiently utilize the soil resources of any given area-through pasture development.

Undoubtedly the greatest limitation to increased legume yield with the cultivars currently available is that of inadequate mineral nutrition. In particular low phosphorus availability limits legume production on a large proportion of soils in most tropical countries (Horrell and Newhouse, 1965; Neme and Nery, 1965; Vincente-Chandler *et al.*, 1964; Franca and Carvalho, 1970; Williams and Andrew, 1970). This aspect will be dealt with in detail in another paper in this workshop so I do not intend to cover the subject in any depth. Phosphorus supply (and for that matter any nutrient deficiency) is intimately associated with nitrogen fixation (Andrew, 1962; Gates, 1970) and, therefore, in most situations we are trading P application for N fixation. Since P is a finite source our interest lies in trading P efficiently and therefore we need to be increasingly concerned with efficiency in the utilization of applied P. There is increasing evidence that legumes differ in their ability to utilize both native and applied P (Blunt and Humphreys, 1970; Andrew, 1973) and this is related to a greater P uptake rather than a lower concentration of P in their tissues. Much of our screening work with tropical legumes has, in the past, been conducted in situations where adequate nutrition has been provided.

As a result it is possible that phosphorus responsive legumes have been selected and those capable of production at lower levels may have been overlooked.

Although the need for calcium by tropical legumes may not be very high, and the dangers of using excess lime have been made clear (Norris, 1958) there may well be scope for using lime in certain situations to increase the yield potential of tropical legumes. Where high exchangeable aluminium and manganese are problems in soils of low pH and low phosphorus availability it may be cheaper to use lime than high rates of superphosphate. Many of the S. American soils are lower in pH and higher in exchangeable Al and Mn than are Australian soils (Bornemisza and Alvarado, 1974) and so the claims made in Australia that tropical legumes do not require lime (Norris, 1958) may not be as universally applicable as was once thought. The whole subject has been admirably reviewed in a recent publication (Norris and Date, 1967). Different soils will have different nutrient requirements to enable maximum legume production to be achieved. The use of critical nutrient concentrations in the legumes grown will aid the assessment of plant nutrient deficiencies and fertilizer requirements (Andrew, 1968).

Rhizobium Associations

In the absence of an effective *Rhizobium* associate tropical legumes fail to compete with other species. However, most of the widely used tropical legumes nodulate freely without inoculation and perhaps this is part of the reason for their success. It is now well recognized that some legumes have specific requirements for inoculation when sown in areas where they are not found naturally and these have been described for the Australian situation (Norris, 1970; Norris and Date, 1976). Failure to inoculate these species with the specific *Rhizobium* will usually result in loss of the legume stand. However, interesting exceptions have occurred in the field. The author has seen stands of *S. guianensis* cv. Oxley, which has a specific inoculum requirement, well nodulated after using the incorrect strain CB756. Although the initial growth was poor, subsequent growth improved greatly (D. Richards, personal communication). In another situation, *Leucaena leucocephala* cv. Peru nodulated in the absence of inoculation. The yields of legume were almost identical to those from treatments which had been inoculated with the highly effective strain NGR8 and serological tests showed that the strain was neither NGR8 nor CB81, the only effective *Rhizobium* strains used on the field station (Date, personal communication). In a third example some *S. guianensis* introductions in the Morphological-Agronomic group 14A (Edye *et al.*, 1976) which did not nodulate with strain CB756 in glasshouse tests, and were not nodulated in the field in the year of sowing, subsequently grew well and were found to be nodulated effectively (L.A. Edye, personal communication). Also some introductions of *S. hamata* failed to nodulate with CB756, were low yielding in the first year, yet by the third year out-yielded

the promiscuous cv. Verano (Edye, *et al.*, 1975). Concerning the legume species generally regarded as promiscuous, Norris (1970) referred to the intriguing problem of determining whether a significant improvement in speed of establishment or vigour of stand could be obtained from inoculation. Subsequent work in Australia has shown that only a small proportion of the applied effective inoculant strain is recovered from nodulated plants in subsequent years, yet the legume stands remain productive (Date, 1974). In Kenya also, Keya and Eijnatten (1975) showed improvement in yield of *D. uncinatum* cv Silverleaf from inoculation up to 20 weeks from sowing in a pot experiment but no advantage when grown in the field.

There is no convincing field evidence that, for perennial legumes that nodulate freely, improved production can be achieved by using applied inoculant strains. Differences achieved in sand culture may not be reflected in field performance because of other limiting factors such as the lower supply of photosynthate in grazed swards or of lower nutrient and water supplies. In one notable example, the most effective strain in sand culture tests became the commercial strain available for *Leucaena leucocephala* - namely NGR8, replacing the strain CB81 previously used in Australia for this species. Field performance was, however, suspect and subsequent field testing on acid soil revealed that NGR8 only formed nodules in the presence of lime (Norris, 1973). Strain CB81 has subsequently been reinstated as the preferred strain for general use with *Leucaena*. The evidence that the nitrogen requirements for higher yielding cultivars are met by the same *Rhizobium* strain suggests that in these situations it is the ability of the plant to fix carbon rather than the ability of the *Rhizobium* to fix nitrogen that prevents increased legume yield. In support of this contention are the results from nitrogen fertilizer studies in which no response to applied N by the legumes siratro, *D. intortum*, *G. wightii* or *T. semipilosum* was obtained in the field (R.J. Jones and P.C. Whiteman, unpublished results). The potential for increased yields in the tropical forage legumes does not therefore appear to be severely limited by lack of suitable *Rhizobium* strains although specific nodulation problems do occur with some legumes which have not been widely used in practice, e.g., Oxley Fine-Stem stylo, and will doubtless occur when species adapted to alkaline soils are grown in soils with low pH or with high levels of available Al and Mn.

With annual tropical forage legumes problems with nodulation in the field have occurred. In only 2 out of 18 trials in northern New South Wales was satisfactory field nodulation observed when the approved *Rhizobium* strains was used at the normal rate (Cloonan and Vincent, 1967). Using 10 to 100 times the normal rate of inoculation improved the situation but did not correct the problem entirely. It is clear that with annual legumes which are growing for a relatively short period, rapid nodulation is essential if high yields are to be achieved whereas the establishment phase is a much smaller proportion of the life history of perennial legumes. Furthermore, it is extremely important that the *Rhizobium* strains used be fully evaluated in the field

before final recommendations on inoculant use are made (Cloonan and Vincent, 1967).

Mycorrhizal Associations

It is now known that endotrophic mycorrhiza occur in some tropical legumes (Possingham *et al.*, 1971).

The presence of these organisms may enable these legume species to grow on soils low in available phosphorus but little is known of the effectiveness of particular host-mycorrhizal associations nor of ways in which such associations may be encouraged for the benefit of the legume. We can expect to learn more about these associations in the future but at this stage the potential for increasing legume yield in low phosphorus situations through the planned use of mycorrhiza is unknown.

Management Practices

In addition to the choice of legume and the control of the inoculation and fertilizer regimes, the farm manager controls several other management practices which may influence the potential yield of the legumes sown. These include the associate species sown, the sowing rates of these species, the method of sowing and the defoliation practice imposed. These will be considered separately.

Associate species

Annual forage legumes are usually sown into cultivated seedbeds and weeds controlled. Failure to control such weeds, which often include grasses from the existing sod, will inevitably reduce the yields of the legume (Murtagh, 1972). Pasture legumes are usually sown with a grass or onto an existing grass stand and the evidence clearly shows that the presence of the grass reduces potential legume yield. In a *D. intortum* cv. Greenleaf - *Setaria anceps* cv. Nandi pasture, mean yields of legume from several cutting treatments were 16.5 t DM/ha in pure stands and 7.3 t DM/ha in the mixture (Riveros and Wilson, 1970). With *Siratro* - *S. anceps* cv. Nandi pasture, Siratro yield depression by the grass varied with cutting treatment but the mean depression was from 4.04 to 2.86 t/ha/yr (Jones, 1974b). Differential effects of the grass associate have been reported. For example, in Hawaii, grasses depressed the yields of associated Centro to a greater extent (compared with pure legume stands) than the yields of *D. intortum* or *D. canum* (Whitney *et al.*, 1967), and the annual *Digitaria ciliaris* depressed the yields of *S. humilis* to a much greater extent than that of *S. hamata* (Torssell *et al.*, 1976). It is also to be expected that grasses differ in their ability to reduce the yields of associated legumes, and Jones (1970) and Olsen and Tiharuhondi (1972) showed that *Chloris gayana* was more competitive with legumes than was *S. anceps*, although in the former

study differences decreased with time. In general the more vigorous grasses may be expected to have the greatest effect in reducing legume yield. However, Kretschmer *et al.*, (1973) reported that reduced yields of *S. anceps*, compared with Pangola or Bahia grass, did not result in yield compensation by the associated legumes.

Among the factors involved in the competition between grasses and legumes the soil fertility status (Jones, R.M., 1975) and the ability of the associate grass to take up potassium (Hall, 1974) are important in determining the productivity of the legume.

Sowing rates

In general high legume seeding rates increase legume yield, and increased grass seeding rates decrease yields of the associated legume (Tow, 1967; Middleton, 1970, 1973; Jones, 1970; Olsen and Tiharuhondi, 1972; Jones (R.M.) 1975). With time the effects of different legume seed rates often decline as the density of legume plants equilibrate with the site and the management imposed (Jones, 1970; Keya and Van Ejnatten, 1975). Thus, for permanent pastures the initial legume seed rates do not greatly affect legume yield in the long term provided the legume species is capable of seeding under grazing or of vegetative spread. With annual fodder crops, however, especially those used in short growing seasons, the seeding rate may be extremely important in achieving high yields.

Method of sowing

In general, legumes establish better than grasses when oversown into existing grasslands (Keya *et al.*, 1972). Conversely, grasses, including weed grasses, often establish better than legumes on well prepared seedbeds where the available N is high, and often their growth in the first year leads to suppression of legume growth (Jones, R.M., 1973). Jones and Jones (1971) have reviewed the literature on methods of establishment of legumes in tropical Australia and conclude that although over-sowing can be successful, its success depends upon the legume species used, the pre-treatment of the existing species and the seasonal conditions following sowing. Early wet season burning of native grassland in N. Australia has greatly reduced native grass competition and increased the reliability of establishment and subsequent yield of oversown Townsville stylo (a shade intolerant species) (Stocker and Sturtz, 1966).

In Malawi, successful establishment of *D. uncinatum* cv. Silverleaf (a shade tolerant species) (Whiteman *et al.*, 1974) has been achieved by undersowing in a maize crop (Thomas, 1975). By undersowing after the maize has been weeded, three to four weeks after sowing, better legume growth has been achieved than by direct sowing although the growth of associated Rhodes grass has been initially poorer under maize.

Defoliation treatment

The yield potential of most tropical legumes can be reduced by inappropriate cutting or grazing management. The trailing and climbing

herbaceous legumes such as *Macroptilium atropurpureum, Desmodium intortum, D. uncinatum, Glycine wightii* (Jones, 1967; Whiteman, 1969; Bryan and Evans, 1973; Olsen, 1973; Jones R.J., 1973a, 1974b.c.) and the shrub legume *Leucaena leucocephala* (Hill, 1971a) are sensitive to frequent cutting or grazing and may be killed if cut low and frequently. Yields of Siratro were reduced by a mean of 225 kg/ha/yr for each week reduction in the cutting interval between 16 and 4 weeks (Jones, 1974b) and the residual effect of the cutting treatments was very pronounced in the year after treatments ceased. In the grazed situation the objective is to produce animal product and not to accumulate legume yield. Some compromise in stocking rate is required to obtain good animal production/ha and yet retain the legume, because legume yield declines sharply with increased stocking rate (Jones, 1974b).

In rotational grazing situations a long rotation would be expected to produce far higher legume yields than a short rotation for these species, and this has been measured on Siratro - *Setaria anceps* pastures (Jones and Jones, in press). It is important to note that the response of these twining tropical legumes to defoliation is so vastly different from the responses obtained with the temperate legume white clover (Jones, R.J., 1967, 1973a, 1974b). However, not all tropical legumes respond in this way. *Stylosanthes humilis* for example can thrive under frequent cutting or grazing and under these situations achieve dominance over associate grasses (Fisher, 1973; Ive and Fisher, 1974). The high altitude tropical legume *Trifolium semipilosum* was also favoured by the low cutting height of 3.8 cm compared with a cutting height of 7.5 cm but gave similar yields when cut every 4 weeks or every 8 weeks (Jones, R.J., 1973b). In the wet tropics, *Desmodium heterophyllum* tolerates heavy grazing and is also productive. Although no experimental data on its performance under different cutting regimes were noted in the literature it would appear to behave quite differently to Siratro and similar twining legumes, and in Fiji it has invaded Siratro-grass pastures which were too heavily grazed for Siratro to persist (I. Partridge, personal communication). In practice, of course, some management practices known to be beneficial for legume persistence and productivity may be difficult to implement, especially on large properties. The problems associated with the use of Townsville stylo pastures in far northern Australia are partly due to the inability to graze the pastures with sufficient stocking pressure during active growth in the wet season to maintain legume density and prevent grass and weed invasion. Furthermore, in some situations such heavy summer grazing on essentially annual pastures may result in severe soil erosion in subsequent seasons (Gillard and Fisher, in press). For these situations legumes which are more competitive with associate grasses and less demanding in terms of grazing management are required; the new releases - *S. hamata* cv. Verano and *S. scabra* cv. Seca - are expected to fulfil these roles from the results of sward tests under a wide range of conditions (Burt *et al.*, 1974; Edye, *et al.*, 1975).

Yields of shrub legumes such as *Desmanthus virgatus* and *Leucaena leucocephala* are markedly dependent upon the cutting treatment imposed

(Parbery, 1967). Not only does the frequent cutting reduce yields but yields are depressed more if cut rather than plucked or grazed (Hill, 1971b; Hutton and Beattie, in press). In the study of Hutton and Beattie, Leucaena produced 7840 kg edible DM/ha/annum with plucking of shoots < 6 mm diameter, compared with 1890 kg from cutting to 30cm. It is probable that the shrubby *Stylosanthes* species now being used experimentally may likewise be adversely affected in comparison under cutting and their yield potential underestimated.

The essential point to emphasise is that legumes differ in their response to defoliation. Failure to recognize this and to manage them according to their specific requirements will result in lower yields of legume or even failure of the legume to contribute sufficient nitrogen to promote a vigorous pasture. Legume persistence is vital to continued productivity of the pasture. Management treatments which prevent the persistence mechanisms of the legume (seed set, seedling regeneration or vegetative spread) from operating, will eventually result in failure of the legume. Management can thus have a very large effect on both the current and on the future yield of legume and of total pasture.

COMPARISONS WITH GRASS YIELDS

Tropical grasses which possess the C_4 dicarboxylic acid pathway of CO_2 fixation (Hatch and Slack, 1970) are potentially higher yielding than species such as temperate and tropical legumes and temperate pasture grasses which possess the less efficient C_3 Calvin pathway of photosynthesis (Ludlow and Wilson, 1970). This higher yielding ability of tropical grasses relative to tropical legumes is due to the higher photosynthetic rates and net assimilation rates coupled with non detectable photorespiration and low intracellular resistance to CO_2. These advantages more than offset their higher dark respiration rates (Ludlow and Wilson, 1972). Since water use from a well-watered sward of grass or legume is similar because the water loss is determined primarily by the environmental factors of radiation, temperature and windspeed (Penman, 1948) it follows that tropical grasses produce more dry matter per unit of water transpired than do tropical legumes and their transpiration ratios (g water/g DM produced) are approximately half those of tropical legumes (Ludlow and Wilson, 1972).

In general, adapted legumes yield only about half as much as tropical grasses grown with nitrogen in the various tropical locations (Colman, 1971). If the values for transpiration ratios quoted by Ludlow and Wilson (1972) of 305-340 for N fertilized grasses and 700 for legumes are used to predict potential legume yields then values of approximately 7,000, 15,000, 21,000 and 28,000 kg legume dry matter should be possible in regions where transpiration values of 500, 1,000, 1,500 and 2,000 mm are measured. Yields under field conditions are generally much lower than these estimates. However, in some situations high yields have been recorded, for example, 12,600 kg/ha in 9 months with *Leucaena leucocephala* in the sub-tropical environment of S.E. Queensland (Hutton and Bonner, 1960), 15,800 kg/ha/annum for *D. intortum* in Uganda (Wendt, 1971),

Table 1. Dry matter yields of some tropical legumes and associated grasses grown in Australia.
(a) Sub-tropical locations.

Species	Yields t/ha/yr Legume	Total	Comments
Glycine wightii cv. Clarence	.53 - 5.30		Annual yields of legume from three cuts followed by grazing. Plots located on a range of soil types.
Desmodium uncinatum cv. Silverleaf	2.40 - 5.47		*Desmodium* superior to *Glycine* on wetter sites.
Lotononis bainesii cv. Miles	2.10 - 3.60		*Lotononis* not persistent when ungrazed.
Macroptilium atropurpureum cv. Siratro	2.20 - 5.30		
Desmodium intortum ex. Hawaii	6.47		
Glycine wightii + *Pennisetum clandestinum* (kikuyu)		7.70 - 8.20	Legume content declined 34 to 60 per cent with age of pasture. January to May yields under rotational grazing with dairy cows. *Colman, Holder and Swain (1966)*
Glycine wightii +	1.90 - 4.10	to 9.60	Mean yields over five years under grazing. Cv. Cooper highest yield
Panicum maximum var. *trichoglume*	1.04 - 4.60		Mean yields over three years. Cv. Tinaroo highest yielder. Mean grass yield 5.16 t. *Edye (1967)*
Leucaena leucocephala cv. Peru	12.60		Dry matter yield over nine months- *Hutton and Bonner (1960)*
Macroptilium atropurpureum cv. Siratro + *Chloris gayana* cv. Pioneer	8.80	17.0	Second season of growth - four cuts. *Hutton (1962)*
Lotononis bainsii + *Paspalum plicatulum* cv. Hartley	1.10 - 3.70	8.40 - 13.7	Range of annual yields over four years.
Macroptilium atropurpureum Breeders material + *P. Plicatulum* cv. Hartley	1.40 - 3.80	4.80 - 13.0	Range of annual yields over five years. *Jones, Davies and Waite (1967)*
Macroptilium atropurpureum Siratro + *P. dilatatum* and *Setaria ancops*	1.60 - 7.30	10.2 - 10.6	Yields are the range for four weekly (lowest yield) to 16 weekly cuts. *Jones (1967)*
	cut / grazed		
Lotononis bainesii	2.60 / 1.40		Legume yields - mean over two years. Grass yields approximately twice that of legumes. Proportion of legume declined under grazing.
Glycine wightii cv. Cooper	6.20 / 2.10		
Macroptilium atropurpureum cv. Siratro	4.30 / 1.10		
Desmodium uncinatum	7.10 / .40		*Whiteman (1969)*
All sown with *Chloris gayana*			
Macroptilium atropurpureum cv. Siratro	8.60		Legume yields in pure swards - means of three years except for *T. semipilosum* (one year). *Evans (1967)*
L. bainesii cv. Miles	5.20		
Glycine wightii CPI 27834	3.70		
Trifolium semipilosum cv. Safari	9.40		
Trifolium semipilosum cv. Safari + *Paspalum dilatatum*	1.21 - 2.43	4.81 - 6.93	Yields are means for four years. Yield variation related to cutting height and cutting frequency. *Jones, R. J. (1973)*

Table 1. *(continued)*
(b) Dry tropical locations.

Species	Yields t/ha/yr Legume	Total	Comments
Glycine wightii + *Cenchrus ciliaris*	0.43 - 1.42	4.14 - 6.46	Mean yields over three years for 26 cultivars: Tinaroo variety highest yield. *Edye et al (1976)*
Stylosanthes humilis + *Urochloa mosambicensis*	1) 2.17 - 3.58 2) 2.71 - 5.21		Mean yields over three years. Erect accessions highest yielding. 22 weeks growing season. Mean grass yield, site 1) 4.94; site 2) 0.48. *Edye and Cameron (1975)*
S. subsericea (5)* *S. hamata* (5) *S. guianensis* (1) *S. humilis* hybrids (2) *S. humilis* (2) All sown with *U. mosambicensis*	1.78 - 2.36 0.99 - 4.29 1.28 1.40 - 2.69 2.39 - 3.11		Mean yields over three years at three sites. *S. hamata* cv. Verano superior in yield at all sites and perennated well. Mean *Urochloa* yield 2.82 t/ha (range 0.93 to 4.85 for sites). *Edye, Field and Cameron (1975)*
S. Guianensis (2)* *S. hamata* (2) *S. humilis* cv. Paterson *S. fruticosa* (3) *S. scabara* (4) *S. subsericea* (3) *S. sundaica* (1) *S. viscosa* (3)	0.22 - 4.07** 0.49 - 4.84 0.70 - 1.19 0.16 - 2.04 2.59 - 7.23 0.37 - 1.25 0.43 - 0.81 0.14 - 4.06		Mean yields over years - ranges over three sites with average rainfalls of 683 - 857 mm. *S. scabra* cv. Seca highest yielding at all sites. Mean yield of volunteer species 0.50 - 3.40 t/ha for lowest and highest yielding sites. *L. A. Edye et al (1976) (in press)* * *No. of accessions tested.* ** *Lowest yield from driest site and highest from wettest site.*
(c) Wet tropical locations			
Centrosema brazilianum *C. pubescens* *C. plumieri*	12.59 10.0 - 11.7 6.80		*C. plumieri* unable to tolerate frequent cutting. Best cool season growth in *C. pubescens* from cv. Belalto. *Grof and Harding (1970)*
Stylosanthes guianensis cv. Endeavour cv. Schofield	3.74 1.96	9.88 9.87	
S. guianensis cv. Schofield		3.90 - 9.0	Legume 53-100%, yield variation due to P rates, cutting frequency and site. *Bruce (1974)*

17,600 kg/ha for *D. intortum* in S.E. Queensland (Riveros and Wilson, 1970) and 20,000 kg/ha/annum with *L. leucocephala* in Hawaii (Takahashi and Ripperton, 1949). Very high yields have been achieved under irrigation in the tropics, e.g., 18,000 kg/ha from *Phaseolus trilobus* and *Clitoria ternatea* in the Sudan Gezira (Musa and Burhan, 1974), 24,000 kg from Siratro, 39,000 kg from *L. leucocephala* and 64,000 kg/ha/annum from *Desmanthus virgatus* in the Ord Valley of N.W. Australia (Parbery, 1967). The remarkable yields from the shrub legumes in the Ord Valley may have been partly due to "Oasis" effects of small irrigated plots in this dry environment. In controlled environment studies leaf photosynthetic rates of 25 mg $CO_2/dm^2/hr$ were recorded by Begg and Jarvis (1968) using *S. humilis* and similar values for a range of tropical legumes have been recorded (Ludlow and Wilson, 1971). From a model describing crop photosynthesis the predicted maximum growth rate of *S. humilis* was 55 $g/m^2/day$ whereas the maximum recorded in the field in Australia is 28$g/m^2/day$ excluding root growth (Begg and Jarvis, 1968).

Yields of legumes achieved in experimental plantings in Australia are given in table 1.

Among the factors which contribute to the generally low yields achieved are the presence of sown and naturalized grasses, the levels of fertilization used, the cutting management and the site x species interaction. Although the growing of pure legume stands has been proposed by Riveros (1970) and would theoretically result in higher legume yields, field experience suggests that such pure stands are more prone to insect attack and soon become infested with weeds. For perennial legumes therefore, mixed grass-legume pastures will continue to be used for grazing and even though the total dry matter yields achieved may be far less than those of nitrogen fertilized tropical grasses, such mixed pastures may be the only viable option available for animal production.

EFFICIENCY OF PRODUCTION

Nutrient Efficiency

The major part of this paper has been concerned with legume yield per unit of land area. Where land is a limiting resource then this is a valid measure of efficient production. In many instances, other factors as well as land area are limiting. Possibly the major factor is that of available nutrients. It then becomes pertinent to measure production in relation to nutrient supply so that yield may then be related to nutrient addition. In such situations, and this applies to most of the natural grasslands in the tropics, it may be more efficient to apply small quantities of nutrients to a larger area than to aim at high yields on much smaller areas. Under these conditions the small amount of legume may have a dis-proportionate effect on animal production because of its supplemental value. To achieve this objective, legumes capable of competing with grasses at low P availabilities and yet

acceptable to stock at critical periods of the year, are required.

In the seasonally dry tropics and where conservation of feed produced in the wet season is impractical or uneconomic, dry matter yield of the legume may be a poor measure of its worth to the grazing animal if the legume is uneaten in the dry season. Leaf fall or fungal growth on the leaves may render the material unpalatable and result in animal production which is poorer at that time than with grass pastures (C.J. Gardener, personal communication).

Seeds and Shrubs

The nutritional value of even herbaceous leguminous pastures declines in the dry season. Seeds, however, are a concentrated source of protein, energy and minerals (Playne, 1969). The retention of seed on legumes during the dry season would therefore appear to be a worthwhile objective in achieving animal production at this time. Photosynthate channelled into seed production would then be utilized more efficiently than if used for stem and leaf production.

Shrubs and trees are known to be less influenced by drought than herbaceous species and hence can provide green feed over a longer period of the year. In spite of these advantages surprisingly little work has been done on the use of such legumes with the notable exception of *L. leucocephala* (Gray, 1970; Hill, 1971b) and even with this species there is very little practical use under grazing.

In the dry areas in particular there is scope for using shrubs and trees for the production of both browse and fallen pods and seeds. The African species *Acacia albida*, for example, is known to produce in edible pods and seeds up to 200 kg/ha of crude protein at low tree densities of 12/ha (Wickens, 1969). This plant also produces leaves in the dry season and sheds them at the beginning of the wet season (Wickens, 1969). The potential in these woody legumes is largely unknown, but the examples quoted should provide sufficient encouragement for further study.

Nutritive Value and Animal Production

Although not within the scope of this paper, it is important in assessing yield potential to ensure that this is not at the expense of feed quality. Animals selectively graze leaf and if forced to graze stem material there is usually a decline in animal performance (Stobbs, 1975). Tropical legumes also differ in digestibility, often widely (Milford, 1967; Jones, 1969) and in their voluntary feed intake when offered to ruminants (Milford and Minson, 1968), although this latter aspect is not well documented. The low voluntary food intake noted for some legumes has been attributed to their trailing habit (Stobbs, 1970), a feature which makes it difficult for cattle to select leaf and, at the same time, achieve a high intake. The low dry weight per unit leaf

area may also aggravate the situation for these legumes since it requires approximately 16 leaves of Siratro or *D. intortum* to yield one gram of dry matter compared with a single leaf of *Leucaena leucocephala* (author's unpublished data).

Low sodium concentrations (Playne, 1970; Jones, 1971; Russell, 1970) and the presence of deleterious substances (Butler and Bailey, 1973) are other factors which need to be considered in assessing the potential of new or improved legumes for animal production.

The final assessment of the forage legume is not solely that of dry matter yield or even of nutrient content but is the contribution made to animal production in a given situation. There is ample evidence to show that where legumes have been used in animal production systems, considerable increases in animal production have been achieved (Hutton, 1970; 1974; Stobbs and Thompson, 1975). Animal performance on these pastures is generally related to the proportion of legumes in the pasture (Bryan, 1970), or to the amount of legume made available to the animals (Norman, 1970). From these results, it may be predicted that higher yielding legumes would result in higher pasture yields, higher carrying capacities and therefore higher animal gains per hectare. However, the relationship may not hold for all legumes and in all circumstances, particularly if the legumes cannot withstand the higher stocking pressure. With our present understanding, assessment of the value of new higher yielding legumes will need to be done in animal production experiments; for legumes already known to have good animal production potential the ability of new high-yielding cultivars to persist under grazing and under the same (or a wider range of) climatic and edaphic conditions than earlier cultivars, may be sufficient.

CONCLUSIONS

The dependence of nitrogen fixation on the yielding ability of legumes emphasises the need for legumes capable of high yields under grazing if high animal production is to be achieved.

Yielding ability is dependent upon the legume genotype, the environment and the management imposed, and large interactions occur.

In the wet tropics and sub-tropics much of the natural variation in legume genotypes has been explored by plant introduction and evaluation and in these areas potential legume yield is currently limited by inadequate nutrition and management rather than by genotype. However, for seasonally water-logged areas and for heavy cracking clay soils, more persistent genotypes are required.

Plant breeding efforts need to be directed towards greater persistence and productivity under heavy grazing pressure with ability to compete more effectively with tropical grasses and with greater cold tolerance for the sub-tropics.

In the dry tropics, and to a lesser extent, the dry sub-tropics, there is much scope for obtaining higher producing legumes than those used commercially. Plant collections from central and south America

have resulted in recent releases of *Stylosanthes* spp. with wide adaptability and potentially high yield. Other material within this genus, and in other genera are currently under evaluation and have promise of further improvements. For these dry situations legumes which establish readily with little or no soil preparation and which can persist and compete against native species with little or no additions of fertilizer are required. Species which produce pods and seeds which are accessible to animals in the dry season may have particular advantages for animal production.

Shrubs and tree legumes have not been exploited to any extent but the results with *Leucaena* and *Desmanthus* are sufficiently encouraging to warrant further study. Ability to grow into the dry season, to use nutrients efficiently and to bear edible pods and seeds are potential benefits, although establishment and management on a large scale could pose problems.

Grazing management has a powerful effect on legume productivity and persistence and there are marked differences between legumes in their reaction to grazing pressure. These different reactions need to be understood if effective utilization of their potential is to be achieved.

Although specific nodulation problems occur with some species, e.g., slow initial nodulation in *Glycine*, *Leucaena* and *S. hamata* (except cv. Verano), in general tropical pasture legumes in the field are not limited by ineffective *Rhizobium* strains.

Relative to tropical grasses well supplied with introgen, the plant growth rates and sward yields of tropical legumes are low, often less than one half. Such a comparison in the practical context is, however, rather meaningless since in very few situations will tropical grasses be able to express their potential because of lack of available nitrogen.

The potential of any pasture legume can only be assessed in terms of animal production. For this reason, yield alone is an insufficient measure of legume potential - the yield must be linked to nutritive quality, the absence of harmful substances and the ability to thrive under grazing.

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AN OVERVIEW OF THE SOILS OF THE ARABLE TROPICS

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INTRODUCTION

It would not be possible to present a balanced overview of the soils of the arable tropics without first probing into the world's projected food requirement and demand. There is a marked difference between food requirement and food demand among the world's richest and poorest nations. In the wealthiest nations demand exceeds food required to sustain adequate human nutrition. In the U.S., for example, 40 percent of the food prepared for school lunches end up as garbage (Mayer, 1975). For many nations, food requirement and demand are more nearly alike, but in times of stress, the poorer nations are not in a position to meet food requirement, as governments and people have insufficient income to buy expensive imported staples.

The world population of four billion people is expected to reach 6-7 billion by year 2000. If we assume no change in per capita food consumption, the world would need to produce half again as much food as it now does. But the problem is not simply to produce more food, which is technically feasible, but to create an economic environment which will encourage increased production in countries where high birth rate threatens to overtake production capacity. Unfortunately, high birth rate and low food production seem to be characteristic of the world's less developed countries so that the forecast for closing the food-population gap appears bleakest for the poorest nations.

The world's poorest nations are largely located in and near the tropics. Of the world's 3.19 billion hectares of potentially arable lands, about half or 1.62 billion hectares occur in the tropics. This area is about equally divided among the humid (30%), sub-humid (36%) and semi-arid or arid (34%) tropics (President's Science Advisory Committee, 1967). Less than half (43%) of the world's potentially arable land is now under cultivation. Large tracts of level land with deep, adequately watered, well drained soils occur in Latin America, Africa, and Southeast Asia. Prominent examples include the Central Plateau of Brazil, the Amazon Jungle, and the Eastern Plains of Columbia, the Congo Basin in Africa, and Kalimantan (Borneo), Sulawesi (Celebes) and Sumatra of Indonesia. Five hundred million hectares of underutilized, potentially arable land are estimated to occur in the humid and sub-humid tropics.

A better population-food-land picture emerges if one examines per capita cultivated land projected for 1985 for selected regions of the world. The per capita cultivated land expressed in hectares per person is estimated to be 0.9 for North America, 0.88 for Russia, 0.34 for Africa and Latin America, 0.33 for Europe and 0.17 for Asia.* The technically advanced countries of Asia, Europe and North America which enjoy high unit area yields are better off or at least as well off in per capita cultivated land as the developing countries of the tropics.

The comparatively low projected per capita cultivated land for Africa, Latin America and Asia and the existence of extensive areas of underutilized potentially arable land, particularly in Africa and South America, point to an uneven population distribution in the tropics. People tend to congregate in places where water and soil fertility are not limiting food production. Conditions of adequate water and soil fertility are met, for example, on the island of Java, on the deltas of the Mekong, Chao Phraya, Irrawady and Ganges-Brahmaputra rivers, on the Pampas of Argentina and in Brazil's São Paulo State. On the other hand, uncertain water supply and aridity near the northern and southern limits of the African tropics keep population density low, and soil infertility prevents people from occupying the well drained, well watered, level lands in Colombia's Eastern Plains, the Amazon and Congo basins, Brazil's Central Plateau and Indonesia's outlying islands.

The statistics indicate that the tropics are blessed with extensive areas of underutilized potentially arable lands, but that they occur some distance from the population centers. Two notable examples of efforts to move people from high density zones to underutilized areas were the establishment of the Federal District in Central Brazil and Indonesia's Transmigration Program. There is little doubt that these efforts would have been more successful had the soils upon which the pioneer farmer found himself been more fertile. A poor farmer can become rich on fertile land, and a rich farmer can make infertile land productive, but a poor farmer on infertile land has little hope of extricating himself from poverty's grip. There are at this moment about two billion people who live in countries with average gross national products of about $200 per capita. In short, there is an abundance of infertile lands and poor farmers in the tropics.

What are these infertile soils like? Is there something fundamentally different about them? What are some of the fertility problems and what can one do about them? These are some of the questions I will try to examine in the next sections.

*Carter, H.O., J.G. Youde, and M.L. Petersen. Future Land Requirements to Produce Food for an Expanding World Population. In "Perspective on Prime Lands." Background paper for Seminar on Retention of Prime Lands, U.S. Department of Agriculture.

Tropical Soils

The term tropical soils is a common name which is used to identify any soil which occurs in the tropics. Like most common names, it lacks precision but is more readily understood by a larger audience than scientific names. This term is frequently used to convey a picture of a red, acid, infertile soil. In the older scientific literature, such a soil was called a Latosol or a Lateritic soil. It is largely correct to say that latosolic or lateritic soils are tropical soils, but the reverse is not true, for there are soils in the tropics which by no stretch of the imagination can be labelled latosolic or lateritic.

For geologically brief periods, soils developed from freshly exposed erosional surfaces, or newly deposited pyroclastics or river sediments are rich in nutrients and support a diverse plant and animal community so long as water and temperature are not limiting. But the nutrient supply is not limitless, and with time and the help of warm and humid conditions, soluble nutrients find their way to rivers and eventually to the ocean. What remains on the landscape is an insoluble residue of highly weathered material stripped of its nutrient supply. These are the impoverished, extensive, underutilized, potentially arable lands of the tropics which continue to intrigue man. Unless there is a reversal in current population trends, the nations that own these lands must eventually learn to use them.

THE NATURE OF WEATHERED SOILS

Are the rules which govern crop productivity different for weathered soils? Most pedologists think not, for rules based on fundamental principles apply everywhere. This question would be academic were it not for the fact that in the current course of events, the flow of crop production technology is from the technically advanced countries of the temperate regions to the developing countries of the tropics. The record of achievement with transferred technology has not been high, and there is a continuing temptation to accelerate this technology transfer in the belief that if a small dose does little good, a lot more might cure the problem.

There is a fundamental difference between the highly weathered, underutilized soils of the tropics and the bulk of the remaining soils of the world. This difference lies in the origin of the surface charge on the soil particle.

The charges on mineral surface arise either from defects (isomorphous substitution of an ion of lower valence in positions normally occupied by an ion of higher valence) in the soil particle, or from surface adsorption and desorption of protons. When the charge arises from internal defects, the defects and therefore the surface charges are permanent and constant. Soil science as we know it is largely based

on a permanent or constant surface charge model.

In old, weathered soils of the tropics, the minerals with permanent charge have been "weathered" out. The remaining insoluble residue derives its surface charge from adsorption of hydrogen ions under acid conditions which leads to positive charges and from desorption of hydrogen ions from surface hydroxyls under alkaline conditions which leads to negative charges. The surface charge can be net positive, net zero, or net negative depending on soil pH. These soils are commonly called soils with pH-dependent charge colloids. There is a natural tendency for the soil pH to drift to a value corresponding to net zero charge. Mattson (1932) referred to this phenomenon as isoelectric weathering.

The fundamental difference between soils with permanent or constant surface charge colloids and those with pH-dependent charge colloids can be shown through the Gouy-Chapman equation

$$\sigma = \left(\frac{2n\epsilon kT}{\pi}\right)^{1/2} \sinh \frac{ze}{2kT}\Phi \qquad (1)$$

which relates surface charge density to surface potential and where:

σ = surface charge density
n = concentration of equilibrium solution
ϵ = dielectric constant of the medium
k = Boltzmann constant
T = absolute temperature
z = counter ion valence
Φ = surface potential

In soils with permanent charge colloids, σ is constant so that manipulation of n (salt concentration) or z (ion valence must result in a change in Φ (surface potential). In the highly weathered soils of the tropics this same change in n and z results in changes in both σ and Φ. In fact if Φ is held constant all of the change must be accommodated by a change in σ. For soils with pH-dependent charge colloids, Φ can be held constant by holding pH constant. The relationship between Φ and pH is given by the Nernst-type equation

$$\Phi = \frac{RT}{F} \ln \frac{H^+}{H^+_o} \qquad (2)$$

where:

R = universal gas constant
T = Absolute temperature
F = faraday constant
H^+ = hydrogen ion concentration
H^+_o = hydrogen ion concentration at the zero point of charge or isoelectric point.

Equation 2 can be rewritten for conditions at 25° C as

$$\Phi = .059(pHo - pH)$$

where pHo is the pH corresponding to the zero point of charge or isoelectric point.

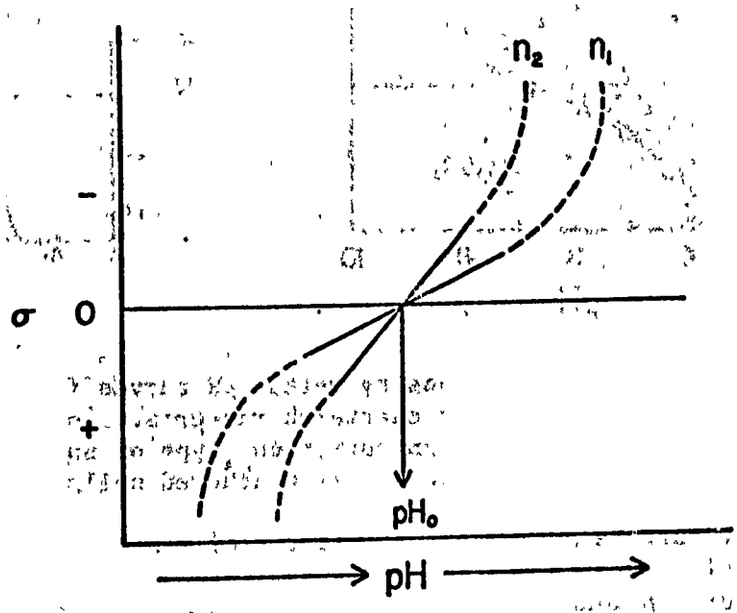
If equations 1 and 3 are combined (Parks, 1967) a relationship between surface charge density and soil pH is developed for the weathered soils of the tropics.

$$\sigma = \left(\frac{n\varepsilon kT}{\pi}\right)^{1/2} \sinh\frac{ze}{2kT}(.059(pHo - pH) \qquad (4)$$

A problem, peculiar to weathered soils with pH-dependent charge clay, becomes apparent when we examine equation 4. In equation 4, as pH approaches pHo, σ approaches zero. This is what Mattson (1932) meant by isoelectric weathering. Since the cation retention capacity (CRC) of a soil is CRC = σS, where S is surface area per unit mass of soil material, the capacity of a soil to hold on to nutrient cations approaches zero values as σ approaches zero. A more detailed account of the cases pHo > pH, and pHo < pH is given by Keng and Uehara (1973).

A graphical representation of equation 4 is shown in figure 1. Experimental curves for soil materials from Latin America (van Raij and Peech, 1972) (Uehara and Keng, 1975), Africa (Gallez, Juo and Herbillon, 1976) and Hawaii (Keng and Uehara, 1973) confirm the applicability of equation 4 to the highly weathered soils of the tropics.

FIGURE 1.



Relations between surface charge density (σ) and soil pH for two soil solution salt concentration ($n_2 > n_1$). The point of intersection represents the zero point of charge (pHo). The dashed portions indicate regions of high buffering capacity. Soil pH normally drifts to pH near the zero point of charge.

FIGURE 2.

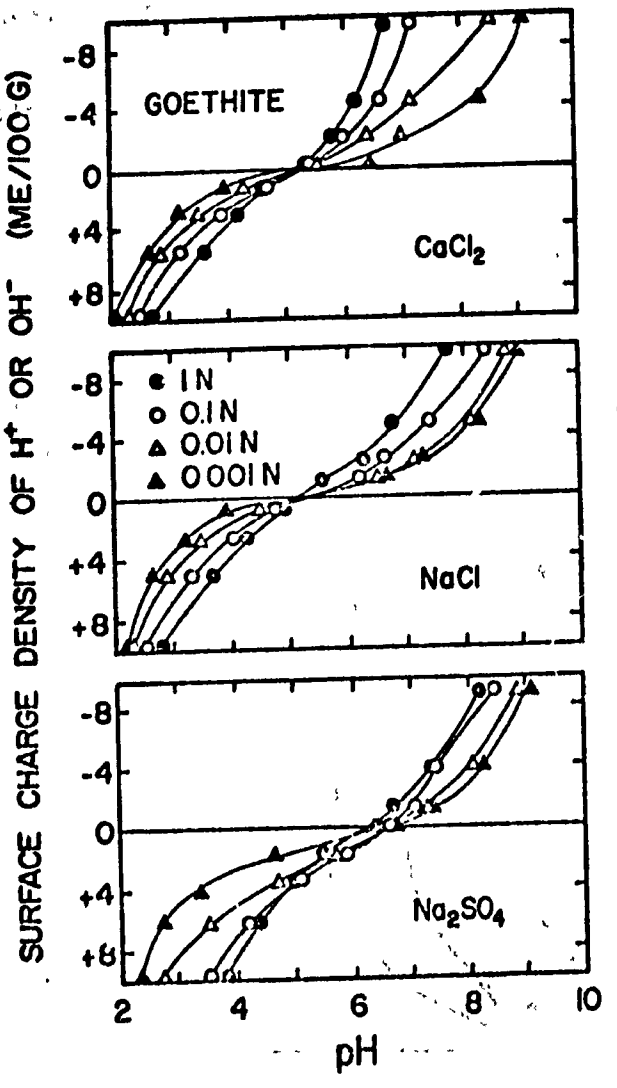
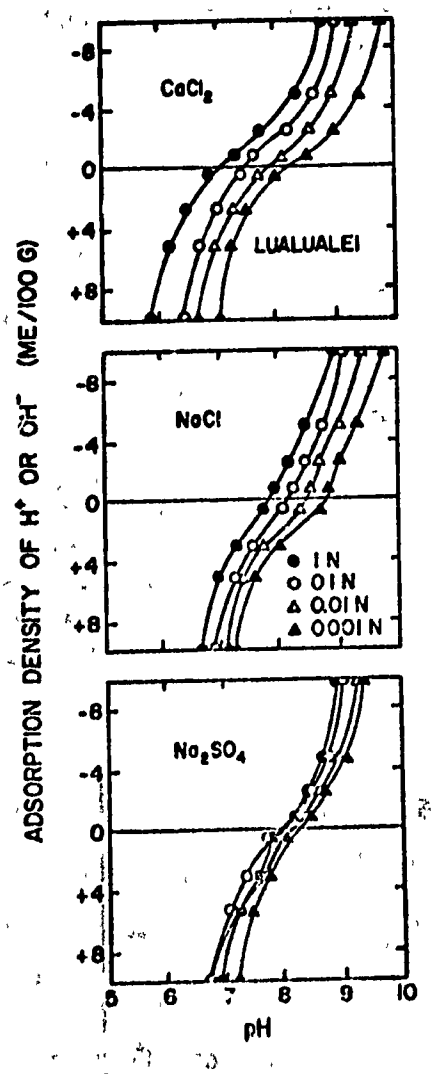


FIGURE 3.



2. Experimental surface charge density versus pH curves for the mineral goethite (FeOOH). The surface charge characteristic of goethite is very sensitive to changes in salt concentration, type of salt and solution pH. Goethite is a common mineral in highly weathered soils.

3. The apparent surface charge characteristic of montmorillonite (smectite). The family of curves do not intersect indicating a constant surface charge. Hydrogen ions are adsorbed but do not create charge in pH range normally encountered in the field.

An illustration of the difference between permanent and pH-dependent charge materials is shown in figures 2 and 3. The data in figures 2 and 3 were generated by adding acid (HCl) or base (NaOH) to soil samples suspended in electrolyte concentrations of 0.001, 0.01, 0.1 and 1.0 N NaCl, $CaCl_2$ or Na_2SO_4. In figure 2, the curves representing various electrolyte concentrations intersect at pHo as predicted in equation 4. The sample in figure 2 is goethite, a hydrated iron oxide commonly found in highly weathered soils.

In figure 3, the curves do not intersect. This implies that the charge on the material is negative and constant. In this case the sample was obtained from a Vertisol, a type of soil noted for its high montmorillonite (smectite) content. Montmorillonite bearing soils are generally young and fertile.

A large majority of temperate region soils and fertile soils of the tropics contain material that react and behave like the mineral in figure 3. In contrast the underutilized lands of the tropics contain materials which are predominantly of the type in figure 2. These differences in the behavior of constant surface charge and pH-dependent charge materials require that man alter his soils management practices as he moves from one system to the other. A large part of current soil management practices will remain unchanged, but an appreciation of subtle differences between these two groups of soil materials will hasten earlier and profitable utilization of the potentially arable lands of the tropics.

CONSTRAINTS TO DEVELOPMENT

Most of the well drained, well watered, underutilized lands of the tropics are occupied by soils with pH-dependent charge minerals. When the time comes, the scientific community must be ready with adequate experience and knowledge to help develop these lands.

Two major soil constraints which stand in the way of easy development of these lands are soil acidity and phosphorus deficiency. We need to assess these two factors in the light of equation 4.

Soil Acidity and Liming

There are several reasons for correcting soil acidity. One important reason is to eliminate or reduce the toxic effects of excessive soluble aluminum and/or manganese. It is now generally agreed (Kamprath, 1970) that aluminum toxicity is, for all practical purposes, eliminated at pH 5.3. If manganese is present in toxic amounts, benefits from lime may occur up to pH 6.5 or higher depending on the crop. In general the first increment of added lime causes a comparatively large increase in soil pH, but at higher pH, the resistance to pH change (buffering capacity) increases. This becomes clear if we take the derivative of σ with respect to pH in equation 4, or look at the slopes of the curve

in figures 1 and 2. The derivative of σ with respect to pH is the buffering capacity of a pH-dependent charge soil material. The buffering capacity is minimum near pH_o and increases rapidly on either side of pH_o. In addition the buffering capacity increases with increasing salt concentration (n) and valence (z) of the liming material. It is obvious from equation 4 that for an equivalent amount of base (OH^-) added to a soil, the pH will be higher with NaOH (z = 1) than with $Ca(OH)_2$ (z = 2)

The problem of soil acidity can be approached in two ways. The first and most obvious way is to apply lime. The second approach is to develop crop varieties which are acid tolerant. A combination of the two approaches offers the best hope for early utilization of highly weathered, acid tropical soils. In some of the most highly leached soils of the humid tropics, lime is needed not so much as an amendment but as a nutrient. The small quantity of lime required to correct calcium or magnesium deficiency may be easily justified, but the same way not be true for correcting soil acidity, so that developing acid tolerant crops should be a major effort for the underutilized, potentially arable lands of the humid and subhumid tropics.

The problem of acid, infertile soils of the potentially arable lands of the tropics is clearly illustrated by the data in Table 1.

TABLE 1

CHEMICAL AND PHYSICAL DATA OF A RED YELLOW PODZOLIC SOIL (UDULT) FROM SUMATRA*

Depth (cm)	Organic Carbon %	Nitrogen %	Extractable Bases (me/100g)				Cation Exchange Capacity (NH4OAc)	pH(1:2.5)		Particle Size (%)		
			Ca	Mg	Na	K		H_2O	KCl	sand	silt	clay
0-10	2.94	0.22	0.3	0.2	0.1	0.2	24.8	4.5	3.8	19.4	35.7	44.9
10-30	1.59	0.14	0.1	0.1	0.1	0.1	26.2	4.5	3.8	16.1	34.4	49.5
30-120	0.79	0.09	0.1	0.2	0.1	0.1	30.0	4.8	3.6	29.3	23.1	47.6
20+	0.35	0.05	0.1	0.2	0.1	0.2	38.8	4.8	3.6	4.0	39.5	56.5

* Data from Soil Research Institute, Bogor, Indonesia

The paradox is that soils like these occur on level terrain, and in regions with adequate moisture and favorable temperatures throughout the year. But without lime and fertilizers, or plants with tolerance to high acidity and low nutrient, these soils will be always categorized as only "potentially" arable.

When soil acidity and infertility are corrected, other problems will arise. During the driest months, crops will suffer from water stress even though the solum is well supplied with water. Unless lime is incorporated into the subsoil, aluminum toxicity will not permit roots to proliferate below the plow layer to extract subsoil moisture. In soils such as these compaction and tillage pans will also prevent deep root proliferation owing to the heavy texture of the subsoil. Loss of topsoil from erosion and the resulting exposure of acid, infertile subsoil can become a serious problem.

Large scale pilot experiments would be highly desirable before governments enter into extensive development of these lands.

Phosphorus Deficiency

The insoluble residue (Primarily iron and aluminum oxide and hydrous oxide) which accumulates in highly weathered soils of the tropics has a high capacity to sorb phosphorus. In heavy textured, high sesquioxide soils, a small application of phosphorus (say 25 kg/ha) may have little beneficial effect on plant growth even though the crop may be severly deficient in that element. In these soils significant responses to phosphorus become evident only after large amounts of the element are added. Fox and his coworkers (1968, 1970, 1975) have studied this problem in detail. From a soil fertility standpoint, phosphorus deficiency and the cost of overcoming it probably present the greatest hurdles to agricultural expansion into underutilized, highly weathered soils.

An example of a soil with high potential for phosphorus fixation is presented in Table II. Unlike the soil in Table I, the soil represented by Table II data is well supplied with bases. Aluminum toxicity is highly unlikely in this soil, but two soil parameters point to high phosphorus fixation capacity. High phosphorus fixation is indicated by the small difference in pH measured in water and KCl solution. Not only are the differences small, the pH in KCl exceeds pH in water in the deepest horizons. When pH in KCl exceeds pH in water, the soil material is net positively charged (Mekaru and Uehara, 1972) and is indicative of a high affinity for negatively charged $H_2PO_4^-$. The material in the surface horizon is net negatively charged (pH in water > pH in KCl solution) only because positive charges have been neutralized with excess negative charge from organic matter. Although organic matter lowers phosphate fixation, high fixation is not substantially reduced by organic matter. High phosphorus fixation is also indicated by high clay content. The clay of such a soil would be rich in sesquioxides - the insoluble residue of prolonged weathering

TABLE II

CHEMICAL AND PHYSICAL DATA OF A RHODIC FERRALSOL (EUTRORTHOX) FROM SÃO PAULO STATE, BRAZIL*

Depth (cm)	Organic Carbon %	Nitrogen %	Extractable Bases (me/100g) Ca	Mg	Na	K	Cation Exchange Capacity (NH4OAc)	pH(1:2.5) H_2O	KCl	Particle Size (%) sand	silt	clay
0-20	1.7	0.15	5.9	2.1	0.1	0.6	14.2	5.2	5.0	28	16	56
20-40	1.0	0.08	4.7	2.1	0.1	0.7	10.0	5.7	5.6	20	20	60
40-60	0.8	0.06	4.2	2.6	0.1	0.3	8.9	6.0	5.9	18	17	65
60-120	0.6	0.05	3.8	2.3	0.1	0.2	7.4	6.1	6.3	23	19	58
120+	0.4	0.03	3.6	3.5	0.1	0.8	8.2	6.4	6.5	44	21	35

* Source: A. van Wambeke (1974). Management Properties of Ferralsols.

under warm and humid climates.

The phosphorus problem becomes less formidable if the long term benefits of phosphorus inputs are considered. Long term benefits involve the residual and amendment effects of sorbed phosphorus. The residual effect of phosphorus has been discussed in a recent paper by Barrow (1976) and is undergoing careful study by R.L. Fox and his group in Hawaii.

The amendment effect of phosphorus fertilizer is only now receiving intensive study. Mekaru and Uehara (1972) and Kinjo (1970) working independently rediscovered what had been known for many years, namely that sorbed phosphorus increases cation retention capacity of soils. Mattson (1931) many years ago had shown that cation exchange capacity of synthetic gels was directly related to silica or phosphorus: sesquioxide ratio.

If we again examine equation 4 we see that phosphorus has its effect on σ and therefore cation retention capacity through pHo. Sorbed phosphorus shifts pHo to lower values and thus makes σ a larger negative value. S.S. Wann * has recently shown that pHo decreases linearly with the square root of applied P.

A similar effect is obtained by applying soluble silica to weathered soils. Syed-Farooq (1972) showed that soluble silica ($CaSiO_3$) or

*Unpublished result of a Ph.D. dissertation. Solute transport in soils with constant potential colloids. Department of Agronomy and Soil Science, University of Hawaii, Honolulu, Hawaii.

phosphorus was effective in reducing leaching losses of potassium, calcium and magnesium from an Oxisol. His findings parallel the earlier work of Ayres and Hagihara (1953) who showed that potassium fertilizer was easily leached when this nutrient was applied as KCl, but was retained in the soil when it was added as K_2SO_4 or K_3PO_4.

These results suggest that the old, weathered soils of the tropics, stripped of their capacity to hold nutrients against leaching, can be rejuvenated by addition of inexpensive silicate amendments. The Brazilians, for example, find it profitable to apply a fused magnesium phosphate fertilizer on highly weathered, high phosphate fixing soils. This fertilizer is manufactured by fusing a magnesium silicate (serpentine or olivine) with low grade rock phosphate. The soluble silica serves to increase cation retention and reduce phosphate fixation. New fertilizers tailored to the special needs of highly weathered soils are needed. The international Fertilizer Development Center in Muscle Shoals, Alabama was created to solve fertilizer-related problems for the developing countries and particularly for the tropics.

The experimental data appear to be telling us that phosphorus and other strongly sorbed anions such as $SO_4^{=}$ and $SiO_3^{=}$ serve as nutrients when they are in the soil solution and as amendment to increase cation retention when sorbed by the soil particles.

SUGGESTIONS FOR FURTHER RESEARCH

Selecting Stress-tolerant Varieties

It is unfair to ask the farmer who has virtually no cash income to grow high yielding, nutritious food crops which require high fertilizer inputs. There are today, technology packages containing seed, fertilizer, and chemicals which enable farmers to double yields, but the high cost of inputs and an uncertain market for farmer produce discourage widespread acceptance of these packages. The farmer can produce more but will do so only if the investment risk is justified.

A maize variety which yields six metric tons grain per hectare when 500 kg/ha of phosphorus is applied, but produces virtually no grain when 50 kg phosphorus is applied, is less desirable than another variety which has a substantially lower yield potential but yields three tons of grain at the lower phosphorus level.

The same can be said for other relations between soil-related stress and crop performance. Other soil-related plant stresses which appear to be critical are soil acidity (aluminum and manganese toxicities), micro-nutrient deficiencies, moisture stress, and extreme temperatures. Successful utilization of the potentially arable lands of the tropics will depend on the extent to which soil scientists are able to economically manipulate soils to accommodate crop requirements and the extent to which plant breeders are able to tailor plants for adverse soil conditions. However trite, it must be said that scientists from both disciplines will have to work together to meet on common ground midway between

wholesale genetic manipulation on the one hand and complete soil alteration on the other.

The soil scientist has an especially important role to play in this regard. He must be able to identify soil-related stresses and express them quantitatively. He must be able to impose and maintain a range of stress in the breeder's test plots.

As an example, if the intent is to select varieties for aluminum tolerance, the soil scientist can help the breeder by selecting a representative acid soil, and by adjusting and maintaining soluble aluminum at a number of specified levels. In this way the genetic pool has a better opportunity for expressing itself fully. When selections are made in this manner, a variety is precisely categorized in terms of its tolerance to a specific soil-related stress and in terms of a well-defined, quantifiable soil parameter.

The most common means of quantifying soil aluminum (Kamprath, 1970) is percent aluminum saturation which is defined as:

$$\% \text{ aluminum saturation} = \frac{Al \times 100}{Al + Ca + Mg + K + Na}$$

in which all metal ions are expressed in milliequivalents per 100 grams soil. Crop varieties which are able to perform well at high percent aluminum saturation are urgently needed for acid tropical soils.

In the case of phosphorus stress, the soil scientist would again locate a suitable soil and adjust soil phosphorus stress to specified levels. It is now generally agreed that for phosphorus, the critical soil-plant parameter is the phosphorus concentration in the soil solution (Fox, *et al.*,1974). The quantity of fertilizer phosphorus one must add to attain a desired level of soil solution phosphorus varies considerably among soils, but is generally very high in highly weathered soils of the tropics. The role of the plant breeder is to select cultivars which can perform well in soils with low soil solution phosphorus, and the role of the soil scientist is to provide the breeder with appropriate stress conditions in the field.

Rhizosphere Biology

In regions of the tropics, where population density is still low and infrastructure is virtually non-existent, every effort should be made to enhance crop productivity through exploitation of rhizosphere biology. An inexpensive alternative to buying and moving huge quantities of high cost, high solubility fertilizer emerges when the fertilizer problem is examined in the light of results obtained with mycorrhizal plants. Rock phosphate is a comparatively inexpensive fertilizer and is frequently more readily available than superphosphates in developing nations. While rock phosphate is relatively ineffective in alkaline and neutral soils, its solubility, and therefore its effectiveness, increases markedly in acid soils. It is now also quite certain that certain fungi *(Endogone)* infect and render roots of higher

plants more efficient scavengers of nutrients, particularly phosphorus (Mosse *et al.*, 1976; Hattingh and Gerdemann, 1975; Tinker, 1975). If we combine the benefits of a cheap fertilizer effective in acid soils, with plant roots infected with mycorrhizae, we have the makings of an inexpensive, low input management system, ideally suited for the infertile, underutilized lands of the tropics.

More efficient utilization of phosphorus fertilizer is not the only benefit derived from the rock phosphate-acid soil-mycorrhizal plant combination. Overcoming phosphorus deficiency will in turn foster more efficient rhizosphere nitrogen fixation.

SUMMARY AND CONCLUSION

About 500 million hectares of underutilized, potentially arable lands occur in the humid and subhumid tropics. The land is frequently flat and the soils deep, well watered and well drained but extremely infertile. Easy and early development of these lands is hindered by high cost of corrective measures.

The underutilized soils of the tropics are either highly acid and leached or highly weathered or both. They are fundamentally different from high base, geologically young soils of the temperate regions. This difference lies in the origin of surface charge on the soil particle. In the young, vigorous soils of the temperate regions, surface charge density is permanent and constant. The surface charge on the highly weathered residue of old tropical soils depends on soil pH, and can be net negative, net zero, or net positive. With increasing weathering and age, soil pH drifts to a value corresponding to net zero charge. Net zero charge represents a point of minimum energy and maximum geochemical stability, but also constitutes an impoverished state from a plant nutrition standpoint.

Lime and phosphorus are two major inputs which are needed to bring the highly leached, highly weathered, underutilized soils of the tropics into full and profitable production. A combination of soil management practices tailored for these soils, a plant breeding program designed to select crop varieties tolerant to stresses peculiar to these soils, and exploitation of rhizosphere ecology offers hope for reducing developmental cost.

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AN ANALYSIS OF THE ROLE OF LEGUMES IN MULTIPLE CROPPING SYSTEMS

A. A. Gomez and H. G. Zandstra

INTRODUCTION

We impose two restrictions that will narrow down the coverage of this paper. First, the symposium title limits our discussion to the tropics where temperature is suitable for crop production all year long. Second, we shall be primarily concerned with farmers whose landholdings are small (5 hectares or less) since most tropical farmers belong to this category (more than 90% of the rice farms in South East Asia) and intensity of land use increases as farm size becomes smaller (Banta and Harwood, 1975).

There are several important considerations in designing adequate multiple cropping systems for small farmers. First, land and not labor is the more limiting resource. In fact, there is usually more labor than can be absorbed by the farm. Second, access to market is poor. It is difficult to procure farm inputs or to sell farm products. Third, cash resource is very limited and cash flow is a very important consideration. Fourth, there is a premium for crops that are edible since they can be used in the household if the market is unfavorable. Thus, for legumes to occupy an important role in the cropping pattern of the small farmers of the tropics, they must be competitive with other crops under the condition described above. In this paper, therefore, we shall discuss the potential of multiple cropping, some characteristics of legumes affecting their use in multiple cropping, environmental requirements of selected legumes, and finally, the important cropping systems involving legumes.

POTENTIAL OF INTENSIVE FARMING

Multiple cropping is an ancient practice and is widespread among small farmers in the developing countries of the tropics. While in the early years, multiple cropping was used to diversify agricultural products to satisfy the needs of subsistent farming, recent experiments have shown its potential for increasing productivity and income among market oriented farmers. Luh (1969), reported that as many as 9 crops per year are raised by vegetable farmers in Hongkong and Vietnam. Bradfield (1973) showed that five crops, including a crop of rice can be harvested in the same area in one year giving a gross return of

$3,150 per hectare. And yet the country averages for cropping intensity in Asia ranges from a low of 1.1 in Burma to a high of 1.84 in Taiwan (Darlymple, 1973).

The potential of multiple cropping is illustrated by the targets for intensifying crop production in the Philippines. Note the wide gap between the intensity obtainable at the experiment station and that actually practiced in the farms. Even the potential under farm condition as shown by experiments in farmers fields are much lower than those in

TABLE 1

PRESENT AND PROJECTED INTENSITY OF CROPPING IN SOME SELECTED AREAS IN THE PHILIPPINES

LOCATION	CROPPING INTENSITY INDEX 1 WITH PRIMARY CROPS 2			
	LOWLAND RICE	UPLAND CROPS	COCONUT	SUGARCANE
	PRESENT (1976)			
Experiment Stn. Trials	4.0	4.0	4.0	2.0
On-Farm Trials	2.5	2.5	2.0	1.3
Commercial Farms	1.6	1.4	1.3	1.1
	PROJECTED (1986)			
Experiment Stn. Trials	5.0	4.0	4.0	2.0
On-Farm Trials	3.0	3.0	3.0	1.5
Commercial Farms	2.0	2.0	1.8	1.3

1 Ratio of harvest area and actual area. For lowland rice and upland crops harvest area for intercrops is the same as pure crops.

2 The most dominant crop of the area.

SOURCE OF DATA: Farming systems commodity team, Philippine Council for Agriculture and Resources Research (PCARR).

the experiment stations. Obviously, there are major limiting factors in the farm that hinder the easy adoption of technology developed at the experiment station.

A major limiting factor for intensive cropping in farmers fields is water. Kanwar (1974) indicated that among the main problems of agriculture in Asia are: erratic distribution of rainfall and the predominance of rainfed agriculture. Because of water limitation many farmers grow only a single crop during the wet months and leave the land idle during the rest of the year.

Agricultural research, however, has evolved techniques for coping with limited water in rainfed areas. First, there are species of crops such as legumes and millets that are tolerant to water stress. Second, selection for earliness has revealed varieties that mature much earlier than the traditional varieties. Third, by modifying cultural management, certain crops can be planted much earlier than before. In rice, for example, by direct-seeding at the beginning of the rainy season instead of transplanting at the peak rainy months, two crops of rice can be harvested in rainfed paddies (Athwal, 1974).

Considering the fact that land is scarce, that farm labor is abundant, and that majority of the low income group is in the rural sector, the intensification of cropping systems is indeed very attractive. Labor absorption per unit area of land can easily be doubled by growing an additional crop. Furthermore, the benefits from intensive land use do not discriminate against, and may even favor, the small landowners. Indeed, the prospects of improved cropping systems is so bright that it could be a means of a more widespread adoption of the green revolution, for bringing the large bulk of subsistence farmers into the stream of market oriented economy, and for solving the serious nutrition problems among the rural poor.

SOME CHARACTERS OF LEGUMES

Advantages

Legumes, especially the annual species, have always been and will continue to be an important component in the cropping patterns of the tropics. This is so because legumes have some special features that make them most suitable for use in intensive farming. Some of these are:

1. Most legumes are very tolerant to drought - Example of legumes known for drought tolerance are mungbean, pigeon pea and soybeans. Thus legumes are generally grown at the end of the rainy season when water stress can be expected at the later stage of plant growth.
2. Some legumes have very short life cycle - Typical examples are mungbean and cowpea which mature in 65 and 75 days, respectively. Most other annual crops like rice or corn take 100-120 days to mature.
3. Legumes are able to fix atmospheric nitrogen - Table 2 shows some estimates of nitrogen fixed by different species of legumes grown

Table 2. NITROGEN FIXED BY LEGUMES IN THE TROPICS

Crops	Kg N/ha/yr	Crops	Kg N/ha/yr
A. From Nutman (1971)1/		B. From Alcantara (1973)2/	
Centro	112	Groundnut	84 - 297
Tick clover	700	Soybean	54 - 369
Stylo	30 - 196	Cowpea	24 - 66
Glycine javanica	160 - 450	String bean	42 - 90
Fenuegreek	44	Mungbean	6 - 32
Lentil	35 - 77		
Cowpea	73 - 240	C. From Mendoza, et *al*, (1975)3/	
Pigeon pea	41 - 90		
Soybean	17 - 124	Ipil-ipil (*L. latisiliqua*	
Guar	37 - 196	cv. Peru	310 - 800
Groundnut	49		
Chick pea	41 - 270		

1/Increase in soil N is primarily from soil analysis and may or may not be due to N fixed by symbiosis.

2/Maturity of all crops under B is less than 4 months and three crops can be grown per year. Figures reported in the literature cited are on a per crop basis. This was multiplied by a factor of 3 to convert them on a per year basis which is comparable to the other figures in the table.

3/Total dry matter N is a product of dry matter yield x % N in dry matter. Thus this value includes soil available N in addition to fixed atmospheric N.

In the tropics. Due to the nitrogen fixing ability of legumes, they do not require as much fertilizer as other crops. As a matter of fact some legumes which are able to fix large amounts of nitrogen are able to increase instead of depleting the nitrogen content of the soil.

4. Legumes have many uses - Many legumes are used in many kinds of foods such as noodles, cakes steaks, soy sauce, vegetables, etc. Leaves of legumes are eaten in some parts of Asia. Legume trunks are good firewood.
5. Legumes have a good market - Leguminous products are easy to sell and command a good price. There are two major reasons for this. First, the product is very versatile and can be used for many purposes. Second, most of the grain legume can be stored at ordinary room temperature allowing the farmers to choose the time when he will sell his products.

6. Legumes have good nutritive value - The protein content in legumes is high. Considering the prevalence of protein deficiency in rural areas, legumes can and should play an important role in the diet of farm households.

Table 3. PRODUCTION IN SOME SELECTED COUNTRIES OF COMMON BEAN *(Phaseolus vulgaris)*

COUNTRY	YEARS	AREA (1000 ha)	AVE. YIELD Kg/ha
Brazil 1/	1970	3,48[illegible]	635
Quatamela 1/	1971	185	331
Indonesia 2/	1971	1,041	700
India 3/	1972	10,310	669

1/From Gutierrez, *et al.*, 1975, as cited by Francis, *et al.*,1975.

2/For soybean and peanut only; from CRIA annual report as cited by Syarifuddin, *et al.*, 1975.

3/For pigeon pea and chick pea only, from FAO production as cited by J.S. Kanwar, 1974.

Disadvantages

Legumes, however, have some deficiencies. Some of these are:

1. Yields of grain legumes are low (Kanwar, 1974; Bressani, 1972) - Average yield of grain legumes from four countries with large legume production are shown in Table 3 and from uniformity trials in South East Asia in Table 4.
2. Many legumes are sensitive to waterlogging - While soybeans and mungbean are tolerant to drought, both are very sensitive to waterlogging. This deficiency is significant in the tropics since water stress due to waterlogging is as frequent as that due to drought.
3. Legumes are sensitive to low pH - The lower limit for mungbean is 5.5 and for other annual legumes 5.0 (Harwood, 1976).

ENVIRONMENTAL REQUIREMENTS OF SELECTED LEGUMES

Cropping systems research results at the University of the Philippines (Los Banos) and at IRRI indicate a considerable range of

Table 4. AVERAGE YIELD (Metric ton/ha[1/] OF THE TWO BEST VARIETIES OF SOME ANNUAL LEGUMES IN SEVERAL COUNTRIES OF SOUTH EAST ASIA

CROP/VARIETY	COUNTRIES Philippines	Thailand	Malaysia	Khymer	Lagos	Average
COWPEA (DRY BEAN)						
All Season	1.77	1.89	1.28	-	-	1.65
UPCA #4	1.70	1.86	1.05	-	-	1.54
PEANUT						
Gedjah	3.31	1.61	0.30	1.99	0.87	1.62
Kidang	3.34	1.59	0.23	1.87	0.77	1.56
SOYBEANS						
Clark 63	1.75	1.66	1.19	2.48	2.82	1.98
TK-5	1.61	0.99	2.03	1.56	1.86	1.61
PEANUT						
CES 87	1.04	1.00	1.75	1.43	0.9o	1.22
EG Glabrous #3	0.79	-	1.61	1.29	0.96	1.16

1/ Average of 2-5 plantings. Computed from 18 m^2 plots.

SOURCE OF DATA: Terminal report, 1970-76. Uniform tests of selected varieties of high protein crops in seven locations in Southeast Asia. SEARCA, College, laguna, Philippines.

rainfall requirements for cowpeas, mung bean, soybeans and peanuts in the Philippines.

Cowpeas showed most versatility of the legumes studied because of the possibility to market as green beans. Cowpeas tend to become indeterminate and yield less during periods of low light intensity, which makes the crop less suited for intercropping into tall crops. On well-drained soils green beans yield from 3 to 6 t/ha can be obtained during periods with rainfall above 100 mm/month. Cowpeas have shown stronger competitive ability against weeds (Moody, 1976) than the other legumes studied. The crop establishes readily after lowland rice and tolerates low tillage management methods. Aside from early damage from flea beetle, insect pests did not reach levels of economic importance.

Mungbeans can best be cultivated towards the end of the rains. They will yield well in the early season but cannot be harvested wet. On a well drained, soil, mungbean yields decreased as rainfall increased (Fig. 1) (Samson, *et al.*, 1976. Mungbeans can tolerate low tillage management in lowland paddy but their competitive ability against weeds is not as strong as cowpeas (Moody, 1976). Mungbean yields after rice varied from 500 to 1200 kg/ha in farmers' fields. Major problems encountered in Mungbean production after rice were crop establishment, early damage due to *Empoasca* leafhopper and flea beetles (Bandong and Litsinger, 1976), and powdery mildew.

Peanuts showed drought tolerance similar to that of mungbeans. The crop can tolerate heavy rain early in its growing period but must receive less than 100mm per month at harvest time to prevent rotting, particularly on finer textured soils. Peanuts are well suited to the post paddy climate, perform better on the less clayey soils and require good tilth. Yields obtained on farmers fields after rice in upland and rainfed lowland conditions varied from 300 to 1200 kg/ha. Peanuts attracted very few pests in 1975 trials. Cercospora leaf spot disease was present but no yield increase resulted from benomyl applications (Bandong and Litsinger, 1976). Peanuts have profitably been intercropped in corn and casava stands.

Soybeans have a short planting season in the Philippines. Unless harvested green, it can hardly tolerate more than 100mm/month of rain during the harvest month. The crop requires at least 10-15 mm/week of rain at flowering time. In Northern Philippines, soybeans planted after November 15 showed greatly reduced yields because of photoperiod sensitivity. This substantially limit the potential for soybeans after paddy rice, particularly where two crops of rice are grown, Soybeans are well suited to seeding after paddy rice in regions where the dry season coincides with longer day lengths such as Indonesia and Malaysia. In the Philippines, substantial yield losses due to flea beetles and beanfly have been identified. Soybeans did not compete well against weeds (Moody 1976) but can readily be established with low tillage by placing the seed directly into the rice stubble.

CROPPING PATTERNS INVOLVING LEGUMES

Legumes in Rotation with Rice

More than half of the rice in Asia is grown in paddies whose source of water is primarily the rain. In these areas there is usually enough rainfall for only one crop of rice per year. Thus, other crops that require less water are usually grown in rotation with rice. Shown in Fig. 2. is a typical rainfall pattern in the Philippines and the cropping pattern that can be used in rainfed paddies.

Legume before rice - Rainfall in many tropical areas, as shown in Figure 1, starts slowly and reaches a peak in about 2-3 months. Traditionally rice is transplanted at the peak rainfall month so that there is available about 2-3 months in which another crop can

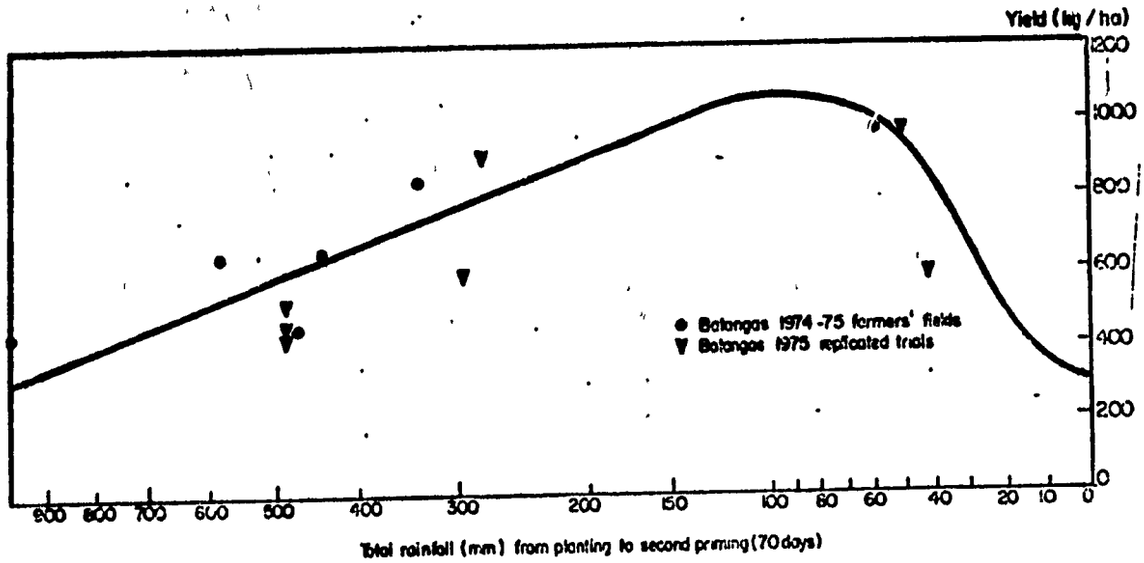

Figure 1. APPROXIMATION OF MUNG BEAN YIELD POTENTIAL FOR BATANGA WITH AMOUNTS OF RAIN FROM PLANTING TO SECOND PRIMING.

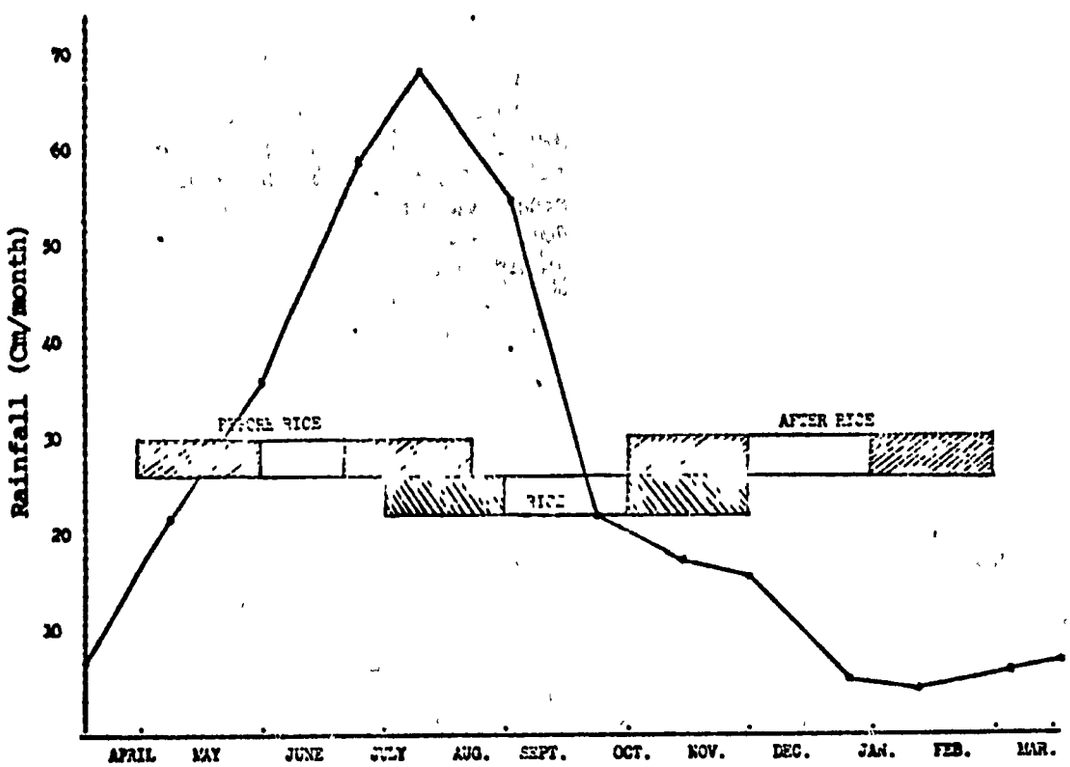

Figure 2. SCHEDULE OF CROPPING PATTERN IN RICE AREAS SUPERIMPOSED OVER A RAINFALL PATTERN.

be grown before rice. There are two requirements for a crop to fit this period. First, it must mature early, preferable in less than 70 days, so that there is enough time to prepare the land for rice. Second, the crop must be able to tolerate heavy rains at the later growth stages. This means a crop that is tolerant to water logging. This also means that the grains do not deteriorate under very wet conditions. Because of these stiff requirements, very few legumes can be grown before rice. Mungbean, for example, which satisfies the early maturity requirement, can not stand heavy rains at harvest time. The few legumes that could be used are cowpea, vegetable soybeans, and other vegetable beans. All these crops can be harvested in less than 75 days and pod deterioration is avoided by harvesting them green.

Legume after rice - Rice is usually harvested at the tail end of the rainy season (Fig. 1) so that the succeeding crop must be able to tolerate drought in the latter growth stages. As indicated in the previous sections, this condition is tailormade for many annual legumes. Thus in many parts of the tropics, legumes are very popular crops in the rice paddies during the dry months.

A major difficulty in growing legumes after rice is in preparing a good seedbed soon after harvest. The conversion from puddled to pulverized well aerated soil is expensive, time consuming and wasteful in terms of residual moisture. To avoid these major difficulties we are looking at the possibility of seeding legumes in uncultivated paddy. In our breeding work, therefore, we are evaluating varieties of soybeans and mungbeans for performance in uncultivated paddy. The results of our first trials are shown in Table 6 and 7. We are pleasantly surprised by the high yields in both species. Most significant is the effect of mulching which substantially increased yield. We are convinced that breeding of legumes for adaptability to uncultivated paddy is productive and are planning to screen many more varieties in the succeeding years.

Intercropped legumes

The distinguishing feature of this pattern is that legumes are grown simultaneously with other crops. This pattern is attractive since the legumes do not complete for sunlight because of their short statute, and for soil nutrients, because of their ability to fix nitrogen. Consequently, there is hardly any yield reduction in the non-legume crop and any yield from the legume intercrop can be looked at as an additional product.

The popularity of legume intercrops is shown by the fact that 98% of cowpea, the most important legume of Africa, is grown in association with other crops (Arnon, 1972) and 90% of the bean *(Phaseolus vulgaris)* crop in Colombia is grown in association with maize, potato and other crops (Gutierrez, *et al.*, 1975 as cited by Francis, *et al.*, 1975). While reduction in yield of the intercropped legume ranges from 18% to 43% (Finlay, 1974b and IRRI, 1973), very little effect is seen in the corn crop (Banta and Harwood, 1975). The value of the total yield of both legume and the non-legume intercrop is almost always higher

Table 5. YIELD AND GROSS RETURN FROM CORN AND MUNG[1] INTERCROP (IRRI 1972)

CROP	UNWEEDED	HANDWEEDED
	Corn yield (mt/ha)	
Corn	2.6	4.1
Corn and mung	3.1	3.3
	Mung yield (mt/ha)	
Mung	1.2	1.3
Mung and corn	0.6	0.7
	Gross returns (P/ha)[1]	
Corn	2600	4100
Corn and mung	4600	5050
Mung	3000	3250

[1]Annual report for 1972, International Rice Research Institute, Los Banos, Philippines, 1973, p. 30.
[2]P=Philippine pesos, approximately US $0.13/peso.

than that from anyone of the monoecrops (Table 5). The increased efficiency of land use obtained by these intercrops generally varies from 30% to 60% above that of the sole crop involved (e.g. Table 5).

Finlay, (1974a) indicated some doubt that the best varieties for mono-cropping are also the best for intercropping. Francis, *et al.*, (1975) suggest that varieties to be recommended for intercropping must be screened separately from those for mono-cropping. We agree with these contentions and have in fact started selecting from among our best varieties of soybeans and mungbeans those that will do well under shade. The results are shown in Tables 6 and 7. While most of the mungbean had very large reduction in yield due to shading, it is interesting to note that two varieties of soybean UPL SY2 and Multivar 80 gave yields that exceeded one ton per hectare. Note further the significant interaction between variety and planting condition which supports the contention that the best varieties for monocrop culture may not be the best for intercropping.

Cropping Systems Involving Perenial Legumes

The perenial legume that has attracted much attention recently is ipil-ipil (*Leucaena leucocephala*). The recent upsurge of interest is primarily due to the introduction of new varieties that are fast growing and high yielding. An interesting work by Mendoza *et al.*, (1975), investigates the possibility of using ipil-ipil as a major component of an upland farming system involving animals, corn and other cash crops. The technique is to grow ipil-ipil in hedge rows that are three meters apart and corn or other cash crops in between ipil-ipil rows. The versatility of this system is shown by the following:

Table 6. BEAN YIELDS IN M. TONS PER HECTARE OF 16 SOYBEAN VARIETIES GROWN UNDER FOUR CONDITIONS, 1975-76 DRY SEASON

VARIETY	LOWLAND PADDY MULCHED	LOWLAND PADDY UNMULCHED	UPLAND OPEN	UPLAND SHADED	MEAN
TK-5	1.645	0.780	1.019	0.743	1.046
Clark 63	2.135	0.910	0.956	0.860	1.215
UPL-SY2	1.495	0.845	1.356	1.020	1.552
L-114	1.760	0.735	-	-	1.247
CES 434	1.580	0.570	-	-	1.075
I. Pelican	1.615	0.750	1.209	0.523	1.024
Wayne	1.240	0.785	0.845	0.673	0.885
#29	1.655	0.660	1.199	0.741	1.063
Americana	0.990	0.480	-	-	1.735
SJ-1	1.525	0.985	0.813	0.543	0.991
SJ-2	1.390	0.630	0.926	0.514	0.865
KE32	1.675	1.100	0.724	0.759	1.064
I-346	1.390	0.730	0.526	0.579	0.806
Bethel	1.535	0.595	1.241	0.986	1.089
Lincoln	1.795	1.395	1.428	0.769	1.346
Tainung 3	1.500	0.790	0.986	0.414	0.922
Tainung 4	1.305	0.920	0.903	0.489	0.904
Williams	2.020	0.845	0.809	0.689	1.090
Multivar 80	1.830	0.300	1.373	1.023	1.131
1248 (Davros)	1.275	0.875	-	-	1.075
MEAN	1.568	0.783	1.019	0.714	1.021
C. V. (%)	15.02	25.50	24.40	17.34	
L. S. D. 0.5%	0.086	.069	0.423	0.217	0.247

1. High yields of dry matter of 9.54-24.07 tons/ha/yr and of crude protein of 1.54 to 5.28 tons/ha/yr were obtained.
2. Ipil-ipil leaves may be left in between rows to serve as fertilizers to cash crops.
3. During the dry season when sun-drying is easy, ipil-ipil leaves can be dried and added to swine and poultry ration. Net return per hectare for ipil-ipil leaf meal production is estimated at $611/ha/yr.
4. The hedge rows of ipil-ipil are potentially capable of reducing soil erosion due to run-off water in sloping areas.

Legumes in Rotation with Other Upland Annual Crops

In contrast to lowland puddled rice, the term "upland crops" refers to all crops that are not grown under puddled conditions. Since legumes and practically all other crops are normally cultivated as upland

Table 7. BEAN YIELDS IN M. TONS PER HECTARE OF 20 MUNGO VARIETIES GROWN UNDER FOUR CONDITIONS, 1975-76 DRY SEASON

VARIETY ENTRY	LOWLAND MULCHED	LOWLAND UNMULCHED	UPLAND OPEN	UPLAND SHADED	M E A N
CES 14	1.048	0.677	1.510	0.433	0.917
CES 28	1.141	0.482	1.214	0.348	0.796
CES 55	1.177	0.670	0.918	0.442	0.801
CES 87	1.030	0.523	1.321	0.374	0.812
MG 50-10A (G)	0.994	0.412	0.958	0.273	0.659
MG 50-10A (Y)	0.936	0.506	1.131	0.348	0.730
EG Glabrous #3	0.853	0.595	1.165	0.351	0.741
DAU MO	1.116	0.583	1.136	0.400	0.808
Bhacti	1.188	0.549	1.156	0.323	0.804
CES Q-1	1.025	0.449	0.569	0.259	0.575
CES U-1	1.067	0.660	1.513	0.328	0.892
CES U-2	0.813	0.331	0.963	0.239	0.586
CES X-10	0.908	0.499	0.863	0.399	0.667
CES Z-10	0.872	0.328	1.176	0.403	0.694
CES ID-1	0.838	0.553	1.191	0.399	0.745
CES ID-21	0.963	0.507	1.376	0.456	0.825
CES 1 BLK-6	0.987	0.611	1.160	0.388	0.764
CES 1E-1	0.846	0.432	0.885	0.313	0.619
CES N-6 (Y)	0.976	0.722	1.471	0.408	0.894
CES 1F-5	0.941	0.563	1.323	0.472	0.824
MEAN	0.986	0.550	1.149	0.368	0.763
C. V. (%)	14.74	17.10	24.40	17.34	-
L.S.D. .05 (%)	.034	N.S.	0.423	0.217	0.043

crops, the type of upland cropping patterns involving legumes is indeed numerous. Their place in upland cropping patterns depends primarily on the rainfall distribution and drainage characteristics of the land. Seeding periods that resulted in acceptable farmers, yields of cowpeas (3.5 - 5.5 t/ha of green beans), mungbeans (0.7-1.5 t/ha), peanuts (0.8-12 t/ha), and soybeans (0.7-1.1 t/ha) in Batangas province were short for soybeans and peanuts, but long for cowpeas (Fig. 2). Because legumes have good tolerance to drought, high nutritive value, and diverse uses, they are probably the most used species in upland cropping patterns.

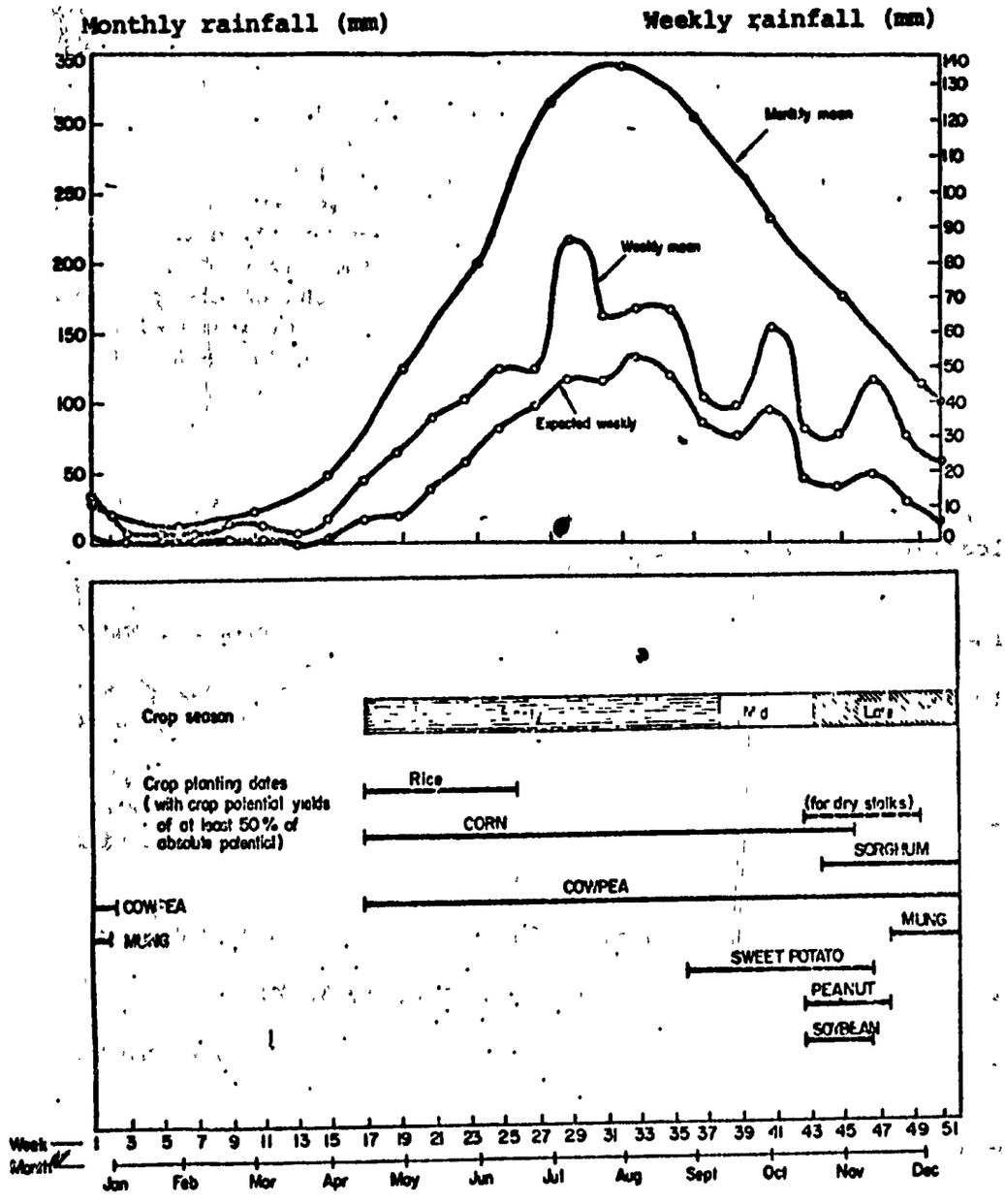

Figure 3. WATER AVAILABILITY AND FIELD CROP PATTERNS FOR UPLAND RICE FARMS OF EASTERN BATANGAS.
(Rainfall recorded at Ambulong, Batangas. Monthly mean rainfall is plotted on the date of the middle of the month).

SUMMARY

Many countries of the tropics are characterized by scarcity of land, abundance of labor, and low income among the farming sector. Under these conditions the prospect of intensifying land use through multiple cropping is most promising.

Because of short their short maturity, tolerance to drought, ability to fix nitrogen and versatile usefulness, legumes have always played and will continue to occupy an important role in the cropping systems in the tropics. The paper presents the environmental requirements of the important annual legumes and finally discusses the following multiple cropping systems: (1) annual legumes in rotation with rice, (2) intercropped legumes, (3) perennial legumes with annual cash crops and (4) legumes in rotation with other upland crops.

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ROLE OF LEGUMES IN SMALL HOLDINGS OF THE HUMID TROPICS OF AFRICA

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The humid tropics is a major climatic zone girdling the earth from 0 - 10° latitude north and south of the equator with extensions of up to 15° north and south of the equator on the western coasts of certain continents. It is characterized by (1) uniformly high temperatures with annual means usually exceeding 25°C, (2) rainfall of over 1,200 mm and sometimes up to 4,000 mm in one or two peaks of the Ar and Aw climates of Trewartha's modification of Koppen's classification of climate (3) precipitation in 6 or more months of the year exceeding evapotranspiration with dry seasons (periods of less than 2.5 cm rainfall per month) of 3 - 4 months, and (4) vegetation ranging from mangrove swamps and moist evergreen forest on coastlines close to the equator to semi-deciduous or dry rainforest in the driest areas adjacent to the tropical savannas.

Smallholders in tropical Africa are farmers whose enterprises are characterized by (1) small farm size of usually 2 Ha or less, (2) labour mainly performed manually by members of the household, (3) use of simple farm tools consisting of hoes, machetes and axes of different types, (4) farming mainly for subsistence but also partially for sale, (5) very little capital and use of low input technology, and (6) marked diversification of production involving several species of crop plants in addition to small numbers of farm animals. The objective of these small farms is not the maximization of production of any single commodity as in modern agricultural systems of Europe and North America but the attainment of stable reasonably good yields of a range of products to satisfy household subsistence requirements with a small surplus for sale to provide ready cash for school fees, clothing, medical expenses etc.

This paper reviews the legumes grown in the humid tropics of tropical Africa, their uses, place in farming systems, nutritional and ethnobotanical inter-relationships in their use and priorities and potentials in modern farming systems based on studies of traditional farming systems in southeastern Nigeria and developments in research at IITA.

Table 1. EDIBLE LEGUMES OFTEN ENCOUNTERED IN SURVEYS IN THE HUMID TROPICS OF SOUTHEASTERN NIGERIA.

CROP	PART UTILIZED	VEGETATION ZONE
Arachis hypogaea	Seeds	Guinea savanna
*Afzelia africana**	Seeds	Tropical rainforest - Guinea savanna
Afzelia bella var. bella*	Leaves	Tropical rainforest
*Brachystegia euricoma**	Seeds	Tropical rainforest - Guinea savanna
*Cajanus cajan***	Seeds	Guinea savanna
*Dialium guinçense**	Fruit	Tropical rainforest - Guinea savanna
*Dioclea deflexa**	Seeds	S. Guinea savanna
*Detarium microcarpiem**	Seeds	Tropical rainforest
Glycine max	Seeds	Subtropics - temperate
Kerstengiella geocarpa	Seeds	S. Guinea savanna
Mucuna pruriens var. utilis**	Seeds	Tropical rainforest
*Mucuna urens***	Seeds	Tropical rainforest
*Parkia clappertoniana**	Seeds	S. Guinea savanna
*Pentaclethra macrophylla**	Seeds & Pods	Subtropics
Phaseolus lunatus	Seeds	Subtropics - temperate.
Phaseolus vulgaris	Seeds & pods	Subtropics - temperate
*Pterocarpus osun**	Leaves	Tropical rainforest
*Pterocarpus santalinoides**	Leaves	Tropical rainforest
*Pterocarpus soyauxii**	Leaves	Tropical rainforest
Voandzeia subterranea	seeds	S. Guinea savanna

* Tree, shrub or perennial.
** Biennial or semi-perennial.

TABLE 2

ESTIMATED CALORIE AND PROTEIN CONTENT OF NATIONAL AGGREGATE DAILY FOOD SUPPLY PER CAPITA IN SELECTED TROPICAL COUNTRIES

Country	Calories (Number)	Total Protein (g)	Animal Protein (g)	Plant Proteins (g)	%
Ghana	2070	43.0	7.3	35.7	83.0
Nigeria	2160	58.6	5.1	53.5	91.3
Tanzania	2140	60.2	12.4	47.8	79.4
Uganda	2160	55.9	15.1	40.8	73.0
India	2000	49.5	6.0	43.5	87.9
Ceylon	2110	45.3	8.3	37.0	81.7
Indonesia	1750	38.2	5.2	33.0	86.4
R. Malasia	2190	49.1	14.7	34.4	70.1
Philippines	2020	48.9	16.1	32.8	67.1
Bolivia	1760	45.8	12.1	33.7	73.6
Brazil	2540	63.8	22.4	41.4	64.9
Colombia	2190	50.1	22.7	27.4	54.7

Source: Palmer, I. (1972). Food and new agricultural technology. UNRISD. Geneva.

LEGUMES

The legumes belong to the second largest family of flowering plants consisting of 600 genera and 13,000 species grouped either in three sub-families, *Caesalpinoideae, Mimosoideae,* and *Papilionoideae* (Faboideae) of Benson (1957), Lawrence (1959) or 3 families *Caesalpinaceae, Mimosaceae* and *Papilionaceae* of Hutchinson (1967 and Hutchinson, Dalziel and Keay (1958). While most members of the Caesalpinaceae and the Mimosaceae are shrubs and trees, most of the Papilionaceae are herbaceous annuals. The family is characterized by the possession of fruits in the form of single carpelled pods or loments and roots with nodules containing rhizobia which are capable of fixing atmospheric

nitrogen. Of the 18 most important or widely grown grain legumes (i.e. *Arachis hypogaea, Cajanus cajan, Cicer arietinum, Dolichos lablab, Dolichos uniflorus, Glycine max, Lathyrus sativus, Lens esculenta, Parkia biglobosa, Phaseolus aureus, Phaseolus lunatus, Phaseolus mungo, Phaseolus vulgaris, Pisum arvense, Pisum sativum, Vicia faba, Vigna unguiculata* and *Voandzeia subterranea)* listed by the PAG (1973) only about 6 species are able to grow readily in the humid tropics.

It should be noted that of the 20 legumes most often encountered in the humid tropics of southeastern Nigeria, 11 are shrubs and trees, 6 are annuals, and 3 are bienniels and/or semi-perennials (see Table 1). Thirteen of these belong to the Papilionaceae, five in the Caesalpinaceae and two in the Mimosaceae.

USES OF LEGUMES

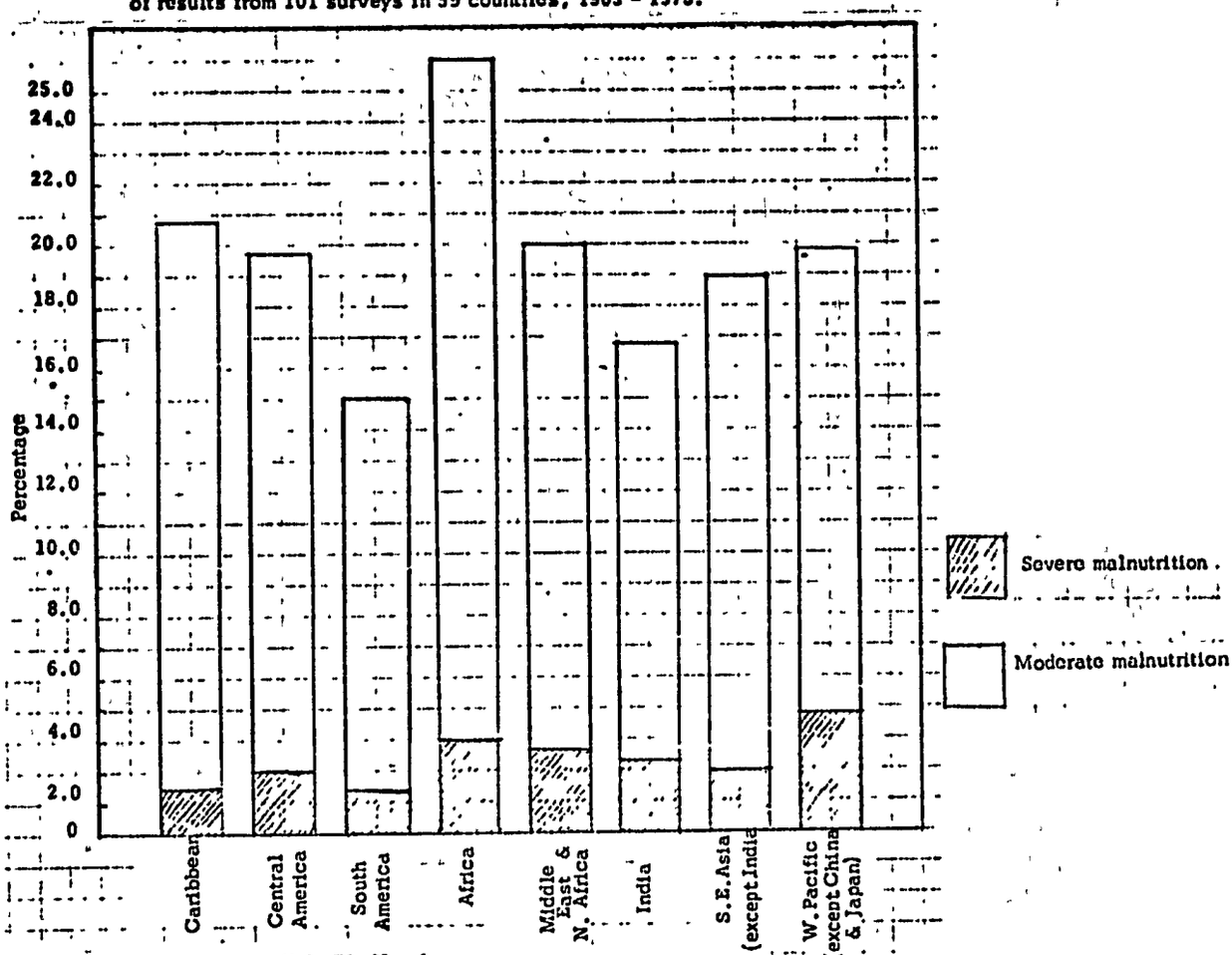
Legumes are important as food crops for sources of proteins of higher biological value than in non-legumes, energy from carbohydrate and fats, minerals, vitamins and roughage. Legumes are also important as animal feed and as sources of miscellaneous useful products such as timber, drugs and oil.

Legumes As human Food

In the humid tropics leguminous food crops are of special significance because of the low protein contents of the major staple food crops consisting mainly of roots, tubers, and cereals, scarcity of animal proteins and consequently heavy reliance of plant proteins in combating protein calorie malnutrition which is rampant in the developing countries of the tropics (see Tables 2, 3 & Figure 1). Plant protein consumption is higher in countries of tropical Africa (73.0 - 91.3%) than in countries of tropical south America, for example Colombia and Brazil (54.7 and 64.9 respectively) in 1972 (Table 2). Legumes in the humid tropics contribute substantially more proteins to the diet than one would expect from the areas of land they occupy or percentage of land under proteins as compared to other crops. Dema (1965) reported that in farms ranging in size from 0.28 - 4 Ha in southern Nigeria, the percentage cropped area under legumes ranged from 1 - 10.8% as compared to 67.7 - 89.4% under starchy staples. In the same area, the supplies of proteins from plants range from 57.4 - 63.8% of which 16.6 - 22.2% or roughly about one third of the plant proteins were from legumes. As shown in Table 4, of the 50 species of crop plants noted for their protein contents 18 (36%) were legumes, 15 (30%) were cereals and 17 (34%) were nuts and seeds of crops from several plant families (FAO, 1976). Protein contents of the legumes are much higher than those of the starchy staples (roots, tubers and cereals).

The quantities of legumes consumed in various parts of tropical Africa vary among areas within countries and among countries in relation to production practices, climate, seasons, customs and cultures and

Fig. 1: Prevalence of severe and moderate protein calorie malnutrition. Compilation of results from 101 surveys in 59 countries, 1963 - 1973.



Source: PAG Bulletin, Vol. IV, No. 1

Table 3. FOOD SUPPLY: Calories and Protein per caput per day from various sources in selected African countries.

Country	Calories per caput per day (number)					Protein per caput per day (decigrammes)		
	Grand total	From roots and tubers	From cereals	From vegetable oils and fats	From pulses	Vegetable products	Animal products	From pulses
Algeria	2138	42	1331	175	31	4547	117	20
Egypt	2637	32	1799	166	76	605	102	57
Ethiopia	1914	57	1352	66	148	497	90	99
Ghana	2318	870	658	146	8	376	158	5
Kenya	2117	203	1195	44	176	482	114	114
Morocco	2661	15	1735	205	81	602	103	108
Nigeria	2085	724	859	181	75	419	44	44
Sudan	2074	206	985	218	35	406	158	198
Tanzania	2008	544	690	69	100	335	136	136
Zaire	1805	1055	307	129	72	242	78	78

Source: FAO Monthly Bulletin of Economics and Statistics 25(4):1-15, 1976.

age (Stanton, 1966). According to Aykroyd and Doughty (1969), results of 97 surveys in 50 areas of 13 countries in Africa south of the Sahara indicated that the amount of legumes consumed per day ranged from 0 - 10 g for 25% of the surveys, 10 - 50 g for 50%, 50 - 150 g (23%) and over 150 g in 2% of the surveys. Seasonal fluctuations in consumption were quite pronounced. For example, in the savanna areas of West Africa daily consumption per capita reached 92 g during the hungry months before harvest when there is usually a shortage of cereals as compared to 30 g after harvest of cereal staples (Aykroyd and Doughty, 1969). In Nigeria average total protein consumption in the savanna areas may be as high as 80 g daily per person as compared to 33 - 40 g in the humid south of which 7.2 g and 4.9 - 7.6 g are from animals in the former and latter respectively (FAO, 1966). In the whole of Africa south of the Sahara average daily intakes of legumes was estimated at 700 g at harvest as compared to an average of 350 g throughout the year. Legumes are not usually fed to children under one year of age (Stanton, 1966). In Ruanda and Burundi daily per capita consumption of legumes among children varying with age were 127 g for 1 - 2 years, 167 g (2 - 5 years), 222 g (5 - 10 years), 296 g (10 - 15 years, 333 g (15 - 19 years).

The most widely grown grain legume in Africa south of the Sahara is *Vigna unguiculata* followed by *Voandzeia subterranea* (Bambara groundnut) *Cajanus cajan* (Pigeon pea), *Phaseolus lunatus* (Lima beans), and *Kerstingiella geocarpa* (earth pea). *Arachis hypagaea* (groundnut) is the most important commercial legume which in many parts of the savanna of West Africa has displaced indigenous legumes such as Bambara groundnut in the traditional farming systems. Other edible legumes which are used mainly for condiments after cooking and/or fermentation include *Mucuna sloanei, Pentaclethra macrophylla, Parkia clappertoniana, Mucuna pruriens* var. *utilis*, and *Dioclea reflexa*. Edible legumes of the humid tropics and adjacent areas are shown in Table 5. Nutritional data on some edible legumes of the humid tropics are presented in Table 6. Although some of the perennial legumes are of minor importance, studies of the time of availability of some of the perennial vegetable legumes such as *Pterocarpus soyauxii* and *Pterocarpus santalinoides* indicate that their edible leaves are only available at critical periods of the year (i.e. dry season) when the conventional sources of annual leaf vegetables are scarce or unavailable (Figure 2). As in temperate legumes, those of the humid tropics such as *Pentaclethra maerophylla* and *Parkia elappertoriana* have to be cooked and fermented to render them nontoxic while *Sphenostylis stenocarpa* and *Cajanus cajan* have to be boiled for long periods of time resulting in use of a lot of firewood or other forms of energy to render them edible.

Legumes as Animal Feed

The humid tropics of Africa is not suitable for rearing of large livestock such as cattle because presence of trypanosomes transmitted by tse-tse flies. However, most smallholders in the humid tropics do keep some goats, sheep, and poultry. Feed for these small livestock may be in the form of browse plants, pasture or cut fodder, kitchen and

Table 4. PROTEIN CONTENT (%) OF GRAIN LEGUMES COMPARED TO VARIOUS NUTS AND SEEDS, CEREALS, AND ROOTS AND TUBERS*

Crops or Plants	% Protein
GRAIN LEGUMES	
Soybean *(Glycine max)*	38.0
Velvet bean *(Mucuna nivea)*	32.8
Nitta tree *(Parkia spp.)*	32.3
Sesban *(Sesbania sp.)*	32.0
Lupine *(Lupinus sp.)*	31.2
Gremsbok bean	27.3
Vetch *(Vicia sativa)*	26.8
Groundnut *(Arachis hypogaea)*	25.6
Sword bean *(Canavalia ensoformis)*	24.5
Lentil *(Lens esculenta)*	24.3
Mung bean *(Phaseolus mungo)*	23.9
Broad bean *(Vicia faba)*	23.4
Cowpea *(Vigna unguiculata)*	23.4
Bonavist niger *(Dolichos lablab)*	22.8
Pea *(Pisum sativum)*	22.5
Bean *(Phaseolus sp.)*	22.1
Pigeon pea *(Cajanus cajan)*	20.9
Chick pea *(Cicer arietinum)*	20.1
CEREALS	
Teosinte *(Euchlena sp.)*	23.8
Sandbur	17.8
Combe fringe	15.7
Job's tears *(Coix lacryma jobi)*	13.8
Oats *(Avena sativa)*	13.0
Wheat *(Triticum aestivum)*	12.2
Buck wheat *(Fagopyron esculentum)*	12.2
Quenoa *(Chenopodium quinoa)*	12.0
Iburu *(Digitaria iburua)*	11.8
Barley *(Hardeum sp.)*	11.0
Rye *(Secale cereale)*	11.0
Millet *(Pennisetum typhoides)*	10.6
Sorghum *(Sorghum vulgare)*	10.1
Maize *(Zea mays)*	9.5
Rice *(Oriza sativa)*	7.5
NUTS AND SEEDS	
Colewort *(Brassica sp.)*	32.0
Baobab *(Adansonia digitata)*	30.0
Deccan hemp *(Hibiscus cannabinus)*	29.9
Chiraulinut *(Buchanania sp.)*	29.3
Panda *(Pandanus aquaticus)*	27.6
Date *(Phoenix dactylifera)*	26.5
Watermelon *(Citrullus vulgaris)*	25.8
Ogokea	24.2
Tropical almond *(Terminalia catappa)*	24.0
Papo-canary tree *(Canarium schweinfeuthii)*	21.6
Manketto *(Ricinodendron rantanenii)*	21.2
Cottonseed *(Gossypium sp.)*	20.2
Hazelnut *(Corylus sp.)*	19.9
Sesame *(Sesamum indicum)*	16.1
Linseed *(Linum usitatissimum)*	18.0
Dried almond *(Amygdalus communis)*	16.8
Walnut *(Juglans regia)*	16.6
ROOTS AND TUBERS	
African yam bean *(Sphenostylis stenocarpa)*	14.6
Water yam *(Dioscorea alata)*	12.0
Three-leaved yam *(Dioscorea dumeotorum)*	10.3
Tumuku *(Solenostemum rotundifolius)*	8.6
Chinese yam *(D. esculenta)*	8.1
White Guinea yam *(Dioscorea rotundata)*	6.8
Berial yam *(D. bulbifera)*	5.7
Risga *(Plectranthus esculentus)*	4.8
Sweet potato *(Ipomoea hatatas)*	2.0
Cassava *(Manihot esculenta)*	2.0
Irish potato *(Solarium tuberosum)*	2.0

*Source for legumes, cereals, nuts and seeds: FAO (1976).

household refuse. In modern livestock production enterprises which are just being developed, cattle and pigs may be feed with feed formulations containing groundnut meal or on pastures and cut fodder with legumes such as *Centrosema pubescens, Stylosanthes guianensis* and *Peuraria phaseoloides* grown sole or usually in mixtures with grasses such as *Panicum maximum* (Guinea grass) *Pennisetum purpureum* (elephant grass). Leguminous browse plants which in addition to nonlegume browse plants are often fed to tethered goats and sheep in the compound farm and fallow systems include *Dialium guineense, Baphia nitida, Millettia* spp., *Erythrina* spp., *Cassia* spp. and *Centrosema pubescens*. Legumes in pastures which may be grazed by animals in the humid tropics include *Centrosema pabescens, Centrosema plumeri, Peuraria phaseoloides, Zornia* spp., *Crotolaria* spp. *Stylosanthes guainensis, Calopogonium mucunoides,* and *Alysicarpus* spp. Leguminous crops whose residues are fed to livestock include *Arachis hypogaea, Vigna unguiculata, Cajanus cajan, Voandziea subterranea* and *Phaseolus lunatas*.

MISCELLANEOUS USES OF LEGUMES

The importance of leguminous plants is not limited to food for man and his animals. Other uses of leguminous plants include (1) soil conservation and fertility maintenance, (2) shade trees and nurse crops, (3) fuel or firewood (4) timber, utensils and various structrual materials, (5) dyes and cosmetics, (6) chewing sticks, (7) ornamentals, hedges and boundary plants (8) medicinals and fishpoisons, (9) games, (10) religious artifacts and related practices.

Soil Conservation and Fertility Maintenance

Legumes, because of their ability to fix nitrogen through symbiotic nitrogen fixation by the rhizobia in their roots, are important in soil fertility maintenance and conservation as (1) components of fallows (2) green manures and cover crops (3) organic manures and mulches etc. (Table 7). In the most widespread method of fertility maintenance in traditional farming systems of the humid tropics is the bush fallow system which relies on natural fallows and vegetation for the recycling of nutrients and recovery of fertility following each cropping cycle. In such fallow the nitrogen fixation in legume components of natural fallows and vegetation makes legumes of special importance in fertility maintenance since nitrogen fertilizers in this era of energy shortage are very expensive for the small farmer to purchase. Unfortunately, no serious studies of the nitrogen fixation and mineral recycling potentialities of perennial legumes as compared to non-legumes has been carried out. Leguminous shrubs and trees such as *Anthonotha macrophylla, Dialium guineense, Afzelia bella* var. *bella, Albizia zygia, Albizia adianthifolia, Baphia nitida, Dalbergia saxatilis, Lonchocarpus cyanescens* and *Millettia thonningii*, were observed by Obi and Tuley (1973) to constitute some of the major plants of bush fallows of southeastern Nigeria.

Table 5. EDIBLE LEGUMES IN OR OF POTENTIAL IMPORTANCY IN THE FARMING SYSTEMS OF AFRICAN HUMID TROPICS*

LEGUME	Status of cultivation			Parts used as food				Other uses and products				
	Wild	Semi-wild	Culti-vated	Leaves	Roots	Fruit	Seed	Oil	Stakes	Forage	Cover crop	Structural materials
Afzelia bella var bella	x	x	x	x			x		x			x
Afzelia africana	x	x					x		x			x
Arachis hypogaea			x				x	x		x		
Brachystegia eurycoma	x	x					x		x			x
Cicer arietinum			x				x		x			x
Canavalia ensiformis		x	x				x				x	
Detarium microcarpum	x	x				x	x		x			x
Digitaria exilis	x	x				x			x			x
Dioclea reticulata	x						x					
Glycine max			x				x	x				
Kerstingiella geocarpa			x				x					
Mucuna sloanei	x	x	x				x					
Lablab niger		x	x				x				x	
Pachyrhizus erosus			x		x							
Parkia clappertoniana	x	x					x		x			x
Pentaclethra macrophylla	x	x	x				x		x			x
Phaseolus lunatus			x				x					
Phaseolus vulgaris			x				x					
Psophocarpus tetragonolobus			x			x						
Pterocarpus osun	x	x		x					x			x
Pterocarpus santalinoides	x	x		x					x			x
Pterocarpus soyauxii	x	x	x	x					x			x
Sphenostylis stenocarpa			x		x		x					
Stizolobium deerningianum	x	x	x				x				x	
Vigna radiata var aureus			x				x					
Vigna radiata var mungo			x				x					
Vigna unguiculata			x	x			x				x	
Voandzeia subterranea			x				x					

Sources: Dalziel (1955); FAO (1959); Keay et al (1964); Rachie and Roberts (1974); Whyte and Trumble (1969); Okigbo (1975).

Table 6. A. COMPARISON OF NUTRITIONAL COMPOSITION OF GRAIN LEGUMES GROWN IN HUMID TROPICS TO MAIZE AND CASSAVA.*

LEGUME	Calories/ 100 gm	Protein %	Fat %	CHO %	Calcium mg/100g	Iron mg/100g	Thiamine mg/100g	Riboflavin mg/100g	Nicotinic acid mg/100g
Kidney bean *(Phaseolus vulgaris)*	341	22.1	1.7	61.4	137	6.7	0.54	0.18	2.1
Cowpea *(Vigna sinensis)*	342	23.4	1.8	60.3	76	5.7	0.92	0.18	1.9
Pigeon oea *(Cajanus cajan)*	343	20.9	1.7	62.9	129	5.8	0.50	0.14	2.3
Groundnut *(Arachis hypogaea)*	546	25.6	43.3	23.4	52	1.9	0.84	0.12	16.0
Maize *(Zea mays)*, 90-96% extraction	360	9.3	4.0	73.5	6	1.8	0.34	0.09	1.3
Cassava *(Manihot exulenta)*	338	1.5	0.6	81.5	12	1.0	9.00	9.00	1.0

B. NUTRITIONAL COMPOSITION OF MISCELLANEOUS LEGUMES OBSERVED IN FARMING SYSTEMS OF S. E. NIGERIA**

	% Protein	Lysine	Tryptophan	Oil	Starch
Detarium microcarpum (wholeseed)	12.0	0.7	0.2	12.0	35.4
Pentaclethra macrophyllum (seed)	20.8	5.0	1.2	45.0	19.0
Mucuna sloanei (shelled seed)	22.4	5.0	0.9	14.4	40.3
Sphenostylis stenocarpa (seed)	23.12	8.2	-	-	51.3
Pterocarpus soyauxii (young leaf)	32.1	0.40	0.5	-	-
Mucuna pruriens var utilis (seed)***	18.6	7.6	2.2	7.3	49.4

* Aykroyd and Doughty (1969. ** Okigbo (1975), Neglected plants of horticultural and nutritional importance traditional farming systems of tropical Africa.

*** Source: FAO Compendium 2:3 (2.3.3/1)

Anthonotha macrophylla also was found to be one of the three dominant species while *Dialium guineense* was one of the abundant species and the rest among the common species. It has for sometime been observed that *Anthonotha macrophylla* is one of the shrubs purposely planted in fallows for maintenance of soil fertility. Other leguminous shrubs used in fallows include *Gliricidia sepium*, *Tephrosia candida* and *Flemminia congesta*. Where cleared bush fallow is stumped, a mixed grass and herbaceous flora develops in which straggling growth of herbaceous legumes such as *Peuraria phaseoloides*, *Centrosema pubescens* (Obi and Tuley, 1973) and sometimes *Calopogonium mucunoides* abound.

Herbaceous legume species some of which grown for forage are also grown for green manuring or cover crops. The green manure crops are usually incorporated into the soil while cover crops are more important in protecting the soil against erosion. Examples of these legumes include *Mucuna prunens* var. *utilis* *Centrosema pubescens*, *Peuraria phaseoloides*, *Stylosanthes guianensis*, *Calopogonium mucunoides*, *Crotolaria juncea* and other *Crotolaria* spp. Leguminous cover crops such as *Peuraria* are regularly used in plantations as cover crops for erosion control and fertility maintenance. Although Moore (1962) at Ibadan, Nigeria, has reported that *Centrosema pubescens* can fix up to 280 kg/ha of nitrogen per year of experiments at Umudike indicated that green manuring was not as effective in the maintenance of soil fertility as the traditional planted fallows with the deep rooted rosaceous shrub *Acioa bateris* as the fallow crop (Obi & Tuley, 1973). It was found that deeper rooted shrubs such as *Cajanus cajan*, *Tephrosia candida*, and *Crotolaria* spp. were inferior to *Tephrosia* and *Acioa* indicating that mineral recycling function of these plants in highly acid weathered and leached soils was more noticeable than the nitrogen fixation. No success has been achieved in getting the farmer to use herbaceous leguminous fallows instead of natural fallows or planted fallows with *Acioa* because of the fact that the advantages of legume covers over natural bush and *Acioa* have not been demonstrated and the several uses of *Acioa* and similar shrubs such as staking and browsing which are not possible with herbaceous and especially creeping legumes.

Residues of legumes used as browse plants constitute part of the farm yard manure made from goat pen beddings and leaf litter of legumes such as *Anthonotha* form humus and organic manure for soil enrichment in fallows. Legumes such as *Stylosanthes* and *Centrosema* are sometimes used in mulching and the superiority of *Stylosanthes* and *Arachis/ Centrosema* mulches over several non-leguminous mulches on maize was demonstrated at Nsukka (Okigbo, 1972).

Shade Trees and Nurse Crops

Leguminous trees and shrubs such as *Albizia ferruginea* and *Gliricidia sepium* have been used as shade and nurse crops in cocoa and coffee plantations. The use of pigeon pea as nurse crop has been reported by Stanton (1966) and the use of leguminous trees (e.g. *Albizia & Saman saman)* for shade for animals in pastures has been reported. The advantages of high shade in acting as wind breaks, reduction of

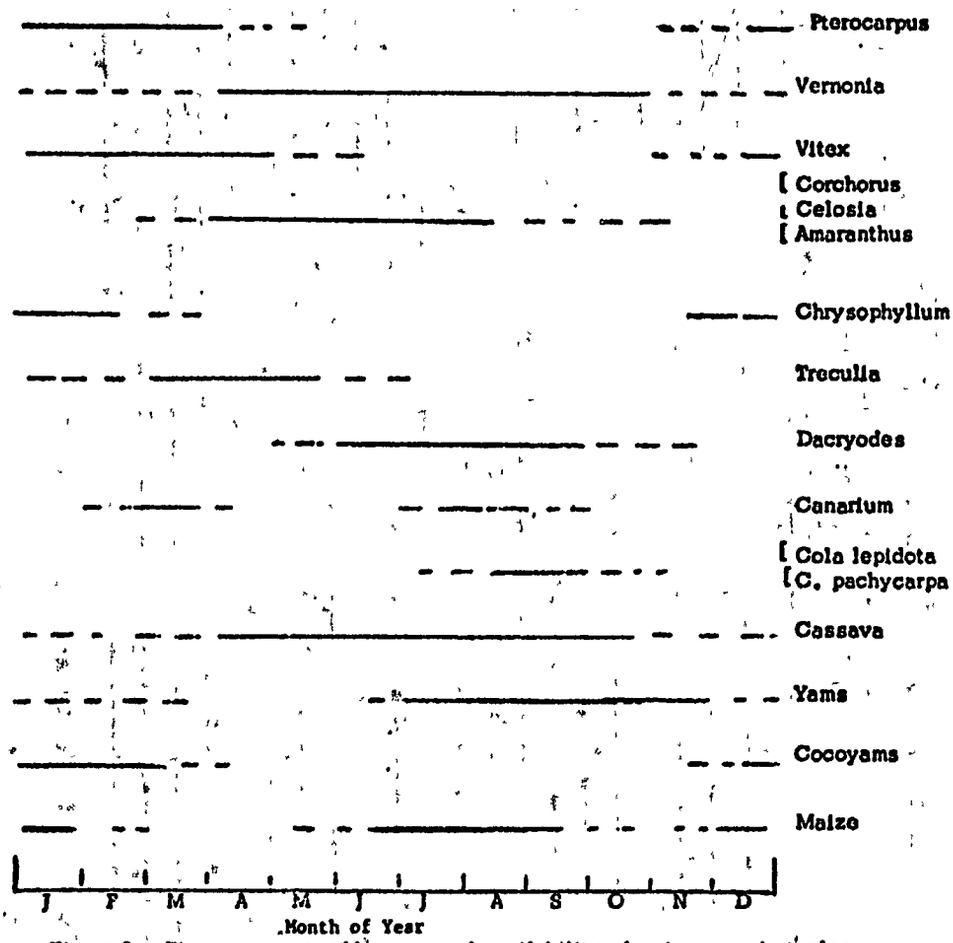


Figure 2: Time sequence of harvest and availability of major annual staples and leaf vegetables as compared to fruit and leaf vegetable perennials found in compound farms in South eastern Nigeria. (Source: Okigbo, 1976)

Table 7. USES OF SOME NON-FOOD LEGUMINOUS PLANTS ENCOUNTERED IN OR OF POTENTIAL IMPORTANCE TO THE LOWLAND HUMID TROPICS

Legume	Uses or potential uses							
	Forage	Cover crop	Green manure	Strip cropping	Mulch	Living mulch	Fallow	Shade
Anthonatha macrophylla					x		x	
Arachis prostrata		x				x		
Calopogonium mucunoides		x	x					
Centrosema pubescens	x	x	x	x	x		x	
Crotolaria spp.		x	x		x		x	
Desmodium triflorum						x		
Desmodium spp.				x	x		x	
Flemingea congesta				x	x		x	
Glycine javanica	x	x	x	x			x	
Glycine wrightii	x	x	x	x			x	
Indigofera spicata		x	x	x			x	
Leucaessa leucocephala	x			x	x		x	
Macroptilium lathyrus			x				x	
Peuraria phaseoloides	x	x	x		x		x	
Psophocarpus palustris		x	x				x	
Sesbania spp.				x	x		x	
Stylosanthes gracilis	x	x	x	x	x		x	
Stylosanthes humilis	x	x	x					
Tephrosia spp.				x	x			
Albizia ferruginea								x
Gliricidia sepium					x			x

Sources: Crowder (1970-71); Whyte and Trumble (1969).

Figure 3. Rows of young maize plants alternating with cowpeas sown through a living mulch of *Desmodium triflorum*.

Figure 4. Rows of maize plants alternating with rows of cowpeas about 8 weeks after planting.

Table 8 : Classification of farming systems of Tropical Africa with indications of the status of associated leguminous, plants of economic and agricultural importance in the Humid Tropics.

Type of Farming Systems in Tropical Africa*	Status of associated leguminous plants
A. Traditional and Transitional Systems	
1. Shifting cultivation (Phase I)	Few cultivated grain legumes especially cowpea may be grown. Most of the legumes utilized for various purposes consist mainly of woody vines, shrubs and some trees in semi-wild condition.
2. Bush Fallow or Land Rotation (Shifting cultivation) (Phase II)	Grain legumes such as cowpeas, lima beans, African yam beans, horse-eye bean, pigeon pea and some groundnuts are grown as minor crops especially close to homesteads. Few are grown on outlying fields. Some legumes, mostly perennial shrubs and trees are protected on fallow land or harvested from the wild.
3. Rudiemntary sedentary Agriculture (Shifting cultivation) (Phase III)	Increasing intensity of cropping with grain legumes grown as minor crops in crop mixtures often occupying specific situations or hedges, fences or close to trees for support and at very wide spacings. Cowpeas, lima beans and African yambeans increasing in importance in outlying farms. Some perennial leguminous food plants still harvested from the wild, others protected or cultivated. Leguminous plants also important components of fallows.
4. Compound farming and Intensive Subsistence Agriculture (Shifting cultivation) (Phase IV)	Grain legumes grown more intensively as vegetables. Most of the perennial legumes protected or semi-wild on fallow land and some cultivated on compound farms.
5. Terrace Farming and Flood Land Agriculture.	On terrace farms, cultivation of grain legumes and othee leguminous species same as on compound farms. Under floodland irrigation, cowpeas and groundnuts may be the only legumes in crop mixtures.
B Modern Systems and their Local Adaptations	
1. Mixed Farming	This system most highly developed in savanna areas; legumes in compound farms and outlying fields. In lowland humid tropics most of the farming systems in Nos. 2-4 are actually mixed farming systems with none of the animals kept for work.
2. Livestock Ranching	Mainly found in the savanna areas where legumes are important natural range plants. Few ranches for beef cattle in humid tropics. Here forage legumes grown alone or as mixtures in pastures with grasses.
3. Intensive Livestock Production (Poultry, Pigs, Dairying)	Forage legumes important even in poultry production in which legumes such as Centrosema may be the main source of carotane, vitamins and minerals. In pig production, forage legumes such as Peuraria may be fed. In dairying, legumes are grown either alone or in mixtures with grasses. (These farming systems are few and but recent developments)
4. Large Scale Farms and Plantations	
a. Large scale food and arable crop farms based on natural rainfall	Grain legumes such as cowpeas may feature as part of the rotation. Soybeans being introduced on experimental basis.
b. Irrigation projects involving crops	Legumes under trial as part of fallow system. Soybean may become important commercial crop in the near future.
c. Large scale Tree crop Plantation	Leguminous trees may be used as shade for coffee and cocoa (e.g. Samanea saman, and Albizia ferruginea). More commonly cover crops e.g. Peuraria grown with the tree crops.
5. Specialized Horticulture	
a. Market gardening	A recent development close to urban centres. Haricot beans, winged beans, lima beans usually grown. Some leguminous plants also grown as ornamentals.
b. Truck gardening and Fruit Plantations	Legumes may be grown as cover crops in commercial orchards such as for citrus or bananas. Enterprises of this kind few.
c. Commercial Vegetable production for processing	Leguminous cover crops may be grown as fallow or green manure. Some may be used as mulching materials.

* Adapted with modifications from Whittlesey, 1936; Morgan and Pugh, 1969; Floyd, 1973; Lant, 1971, Greenland, 1974; Benneh, 1974; Okigbo, 1975.

temperature ranges, maintenance of humidity within the crop, removal of excess rain water, reduction of weeds, reduction of pests and disease incidence, maintenance of soil organic matter and structure etc. and its disadvantages in reduction of light intensity and response to fertilizer, competition for nutrients, acting as alternate hosts for pests and diseases etc. have been reviewed by Wrigley (1969).

Fuel and Firewood

The most important use of trees and shrubs in the humid tropics is that of fuel and firewood for heating and cooking. Many of the leguminous trees and shrubs are sources of firewood. Some legumes such as *Dialium guineense* are good sources of charcoal used by local blacksmiths and recently for barbecuing. The large woody pods of leguminous trees such as *Pentaclethra macrophylla* in addition to the wood from the same tree make excellent fuel. Some quick maturing legumes e.g. *Cassia siamea* are grown mainly for fuel.

Timber and utensils

Among the numerous uses of legumes are those of structural materials and timber. Important commercial timbers of the humid tropics which are legumes are *Brachystegia eurycoma, Pterocarpus osun, Afzelia africana, Parkia bicolor, Berlinia sp.* etc. *Pentaclelthra macrophylla* and *Dialium guineense* are used in making of tool handles and durable structural materials such as pillars for buildings in addition to the making of utensils such as mortars, pestles, wooden spoons and forks.

Dyes and Cosmetics

Leaves of legumes such as *Phaseolus lunatus, Vigna unguiculata* and *Mucuna sloanei* are used for colouring and beautifying mud walls, floors and sometimes masks. The indigo dye of widespread use in local textiles is mainly from *Lonchocarpus cyanescens* (Yoruba indigo) which is often cultivated or protected for this purpose. The roots of Cassia alata are used in tatoolike skin dyeing while the wood of either *Baphia nitida* or *Pterocarpus soyauxii* yield camwood which is ground to produce an orange red dye used for cosmetic purposes.

Chewing Sticks

In West Africa, woody tips of twigs of trees are chewed and used as tooth brush. Leguminous shrubs or trees used for this purpose include *Baphia nitida, Dialium guineense* and *Millettia* spp.

Ornamentals, Hedges and Boundary plants

Many leguminous trees and shrubs are grown as ornamentals because of the aesthetic characteristics of their flowers and other structures. Legumes of the tropics commonly grown as ornamentals include - *Cassia* spp. (e.g. *Cassia nodosa* and *Cassia siamea), Bauhinia* spp., *Canavalia ensiformis, Clitoria* sp., *Albizia* spp., *Delonix regia, Abrus precatoris, Baphia nitida, Tephrosia vogelli* and *Erythrina* spp. Leguminous shrubs whose branch cuttings and twigs sprout easily are often used for hedges

and fences for keeping out animals from gardens and for boundary plants. These include *Baphia nitida, Gliricidia sepium, Albizia* sp., *Erythrina* spp, *Pterocarpus* spp., and *Milletia* spp.

Medicinals Drugs plans, Fish poison Insecticides

Many leguminous plants found under cultivation or in the wild in the humid tropics are used in folk medicine or as sources of drugs and insecticides. Examples of these include *Abrus precatorius* (root & bark used as emmenagogue), *Baphia pubescens* (bark - essential oil santalol), *Cassia* spp. (fruit - anthraquinone glycosides, sennasides A & B), *Erythrina senegalensis* (seeds - alkaloid hypaphorine and others), *Lonchocarpus cyanescens* (bark and leaves - rotenone, lonchocarpine), *Physostigma venenosum* (seeds - alkaloid physostigmine, eserine, eseramine physovenine, gereserine, phytosterols, stigmasterol, sitosterol), *Pterocarpus erinaceous* (bark-resin, kino), *Baphia nitida* (wood-iso-santalene), *Cassia alata* (leaves-saponin), *Dialium guineense* (leaves-fibrifuge, diarrhoea), *Tephrosia vogelli* (leaves-saponin, tephrosin, deguelin), and *Tetrapleura tetraptera* (bark-fruits-mimosine, saponin), Oliver (1960). Plants of the *Tephrosia spp.* are sometimes grown in compound farms for use as fish poison.

Games

Seeds of legumes are sometimes used for playing games for example *Ceasalpinia bonduc* and *Mucuna sloanei*.

Religious artifacts, fetish and Related Uses

Several species of legumes are sources of traditional religous artifacts or are grown and/or used as fetish in sacred groves. These include *Baphia nitida, Pterocarpus osun, Pterocarpus soyauxii, Detarium microcarpum, Voandzeia subterranea* and *Entanda pursaetha*.

Ornaments and Necklaces

Legumes are used for decorative purposes by using their seeds in beads and necklaces. Examples of legumes used for this purpose include *Detarium microcarpum, Ceasalpinia bonduc, Entanda pursaetha* and *Abrus precautoris* (Dalziel, 1936).

NUTRITIONAL AND ETHNOBOTANICAL INTERACTIONS IN UTILIZATION OF LEGUMES

The complex inter-relationships in the utilization of legumes and non-legumes in the humid tropical areas of Nigeria can be illustrated with the two perennial legumes, *Anthnotha macrophylla* and *Dialium guineense* which are found in abundance in traditional bush fallow systems.

Anthonotha macrophylla has found widespread use as a planted fallow shrub in southeastern Nigeria. In addition to its use as a fallow legume which probably enriches the soil with nitrogen fixed in its roots, minerals recycled through leaf fall and organic matter

added to the soil, there are many other uses unrelated to soil fertility. During bush clearing, the small branches are heavily pruned and the main branches 2 - 3 meters high are left standing to be used as stakes for climbers such as cowpeas, yams fluted pumpkins, common pumpkins, white melon, yam beans and horse eye bean. The lopped off branches, dead stems and stumps are used as firewood. Leaves of *Anthonotha* are usually used in the wrapping and fermentation of castor beans used as condiment in soups and they impart a desirable flavor to the product. Stumps of old plants during weeding operations are used for the desication of difficult-to-kill weeds such as *Portulaca* spp, *Talinum triangulare, Commelina* spp, and *Cyperus* spp. which are hung on the stumps to facilitate their drying without the development of new roots. The leaf litter of *Anthonotha* as it decomposes supports growth and development of a special mushroom which is highly relished in the area. The shrub is also grown as an ornamental in gardens and parks.

Dialium guineense (velvet tamarind), though abundant in bush fallows is often protected and cultivated for its fruits. The aril around the seeds are usually relished at certain times of the year (February - May). It is also a browse plant that is liked by goats and sheep. Unlike *Anthonotha* whose wood is very susceptible to powder post beetle damage, *Dialium* wood is very durable and is used in carving of utensils (mortars and pestles), tool handles, combs, pillars, etc. Twigs of *Dialium* make very good chewing sticks. Charcoal from velvet tamarind is valued by blacksmiths as well as the wood for cooking. In addition to its used for staking as with *Anthonotha*, velvet tamarind is sometimes grown as an ornamental.

Thus there are complex inter-relationships in the utilization of both *Anthonotha* and *Dialium* which relates them to the farmers, their environment and culture by the association of their use with the utilization by man of other plants. No annual legume fulfils so many functions as do some leguminous shrubs and this enhances the status of perennials in traditional farming systems as indicated in Table 8.

LEGUMES IN FARMING SYSTEMS OF THE HUMID TROPICS

Most farms operated by smallholders in the humid tropics consist of more or less centrally located compound farms which are intensively cultivated with annual staples and many perennial crops in mixed culture at varying distances from the homestead. From these are located in different directions farms consisting of different field systems with varying periods of fallow and intensities of cropping. The status of useful legumes associated with these farming systems include wild, semi-wild or protected and cultivated plants. The wild useful plants are often found in farms located far away from the farms and are left in fallows or only partially burned during clearing operations. Useful plants in this category include *Parkia clappertoniana, Dialium guineense, Pterocarpus santalinoides, Tetrapleura tetraptera, Afzelia africana* and *Abrus precatorius*. The semi-wild protected edible or useful leguminous

plants are those which are abundant or dominant in areas of short duration fallows where they are protected or left to grow as volunteers and are rarely completely cut off during bush clearing. Examples of legumes in this category are *Pentaclethra macroplylla, Cassia alata, Pterocarpus soyauxii, Parkia clappertoniana, Dialium guineense, Dioclea deflexa, Afzelia bella* var. *bella*. Cultivated legumes are those leguminous plants under regular cultivation in farms of the humid tropics, for example, *Vigna unguiculata, Sphenostylis stenocarpa, Voandzeia subterranea, Cajanus cajan, Phaseolus lunatus*, etc. Some legumes found in the three categories (wild, semi-wild and cultivated) include *Pentaclethra macrophylla*, and *Pterocarpus soyauxii* while *Cajanus cajan* (Pigeon pea) may be found as cultivated and as semi-wild or protected plants.

Legumes in Cropping Systems

Most of the herbaceous leguminous grain legumes in the humid tropics are high risk crops because of their susceptibility to diseases, insects, nematodes and other pests. For this reason they are grown as minor crops and usually as intercrops with major staples. It is also common practice in both compound farms in which most of the grain legumes are grown as vegetables and in bush fallow farms located at varying distances from the household to find legumes grown in strategic positions along fence lines, walls, hedges, close to stakes for yams and at the bases of tree crops such as *Pentaclethra macrophylla*. Thus they are located where there is existing support and along the edges of farms where pods of legumes such as *Vigna unguiculata* can be harvested green without penetrating deep into the farm or garden.

The role or status of various legumes in the farming systems of tropical Africa are summarized in Table 8, taken from Okigbo (1975).

Potential uses of legumes in Farming Systems of the humid tropics

In the improved farming systems of the tropics, legumes will still play a vital role as foodgrains, forages, cover crops, green manures, live mulch, etc. Current research at IITA, for example, indicate that legumes can be used in minimum tillage to provide soil cover when the legume is grown as a fallow crop for two or more years, then killing it with herbicide which turns it into dead mulch in which is sown maize, cassava, and cowpeas without further cultivation. Legumes found promising in this way include *Pueraria phaseoloides, Stylosanthes guianensis, Psophocarpus palustris* and *Centrosema pubescens*. There is also the possibility that some legumes can be grown as live mulch (i.e. as living cover) through which crops such as maize, rice, cowpeas and soybeans can be drilled without cultivation or killing of the legume cover (see Figures 2 & 3). Under such conditions the legume contributes nitrogen to the soil and with phosphorus, potassium and very little nitrogen added, maize yields of up to 2.38 t/ha on live mulch as compared to 2.23 t/ha on ordinary crop residues and rice yields of 4.12 t/ha on live mulch as compared to 3.41 t/ha on dead rice residue cover have been obtained (Wijewardene, 1976).

GENERAL OBSERVATIONS AND FUTURE POTENTIALITIES AND PRIORITIES OF LEGUMES IN THE HUMID TROPICS

General Observations

From the foregoing the following general observations can be made:

1. Legumes are minor crops in the farming systems of the humid tropics but the few legumes grown make substantial contributions to the protein supplies of people in the humid tropics.
2. There is still limited knowledge about the yields of the various edible parts of more than 50% of the edible legumes of the humid tropics.
3. There is limited knowledge and research on the nitrogen fixing potentialities, inoculation requirements and soil fertility maintenance potentials of legumes in the humid tropics especially the perennial legumes.
4. Although as indicated above legumes are minor crops grown in mixed culture in farming systems of the humid tropics, they are put to miscellaneous uses and are of strategic importance in the nutritional well being of the people in the humid tropics.
5. Some edible legumes of the humid tropics have to be detoxified by cooking, fermentation and other practices to render them less toxic before they can be eaten.

Future Research

To realize the full potentialities of legumes in the farming systems of the humid tropics priority should be given in research to the following in the immediate future:

1. Nitrogen fixation studies and use of legumes in soil conservation and fertility maintenance.
2. Study of cropping combinations, sequences and rotations that take advantage of nitrogen fixing potentials of legumes in continuous cropping systems.
3. Improvement of yields and adaptation to various environmental conditions and stresses.
4. Study of food values, toxic substances of various edible parts and their elimination.
5. Processing and storage studies.
6. Studies aimed at deversification of uses and production of legume.

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LEGUMES IN THE SEMI-ARID TROPICS

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INTRODUCTION

The semi arid tropics (SAT) are characterised by an arid season lasting 5-10 months. More than 95% of the rainfall of 500-1500 mm occurs in the 4-7 months rainy season. Rainfall is undependable and probably the major constraint to production. SAT zones occur in 48 countries, with a total area about 19.6 million km^2 and a population over 500 million people, over half of whom live in India.

ICRISAT was established in 1972 and among its objectives are Improvement of the major SAT crops - millet, sorghum, groundnut, chickpea and pigeonpea, and Development of farming systems to stabilise and increase production.

Cereals dominate SAT grain production, with about 43.7 million t of sorghum and millet on 67.6 million ha *cf.* 30.8 million t of grain legumes on 47 million ha. The production of pulses, particularly of chickpea, has declined in India by 21% since 1964-65. The average yields in India of sorghum (547 Kg/ha) and millet (397 Kg/ha) are still less than that of groundnut (709 Kg/ha), pigeonpea (677 Kg/ha), and chickpea (580 Kg/ha). Yields can be readily doubled by good management. Farm sizes in the SAT regions of the less developed countries are small - for India 90% of holdings are less than 6 ha. Virtually no fertiliser is applied to SAT legumes.

There is very little cultivation of legumes other than for grain, with SAT groundnut production the largest at 12.2 million t, or up to two thirds the world production. India is the largest producer with 5.0 million t. In Africa, Nigeria and Senegal are the major producers. Yields are limited by *Cercospora* leaf spot diseases and rust. With disease control, yield potentials are very high; 7000 Kg/ha nuts in shell has been obtained under rainfed conditions. Little response to inoculation has been obtained.

Chickpea is the major pulse legume, with 5.8 million t produced on 9.5 million ha. 74% of this production occurs in India, followed by 10% in Pakistan, and 4% in Ethiopia. Two types of chickpea are grown - the small seeded Desi mainly in the Indian subcontinent and Ethiopia, and the large seeded Kabuli type in West Asia. These two types have probably been separated for thousands of years, and crosses between them at ICRISAT show much promise for increasing yields. The crop is

usually grown as a sole crop in winter in India, Pakistan, Ethiopia, Sudan, Mexico and Chile but elsewhere as a summer crop. In India chickpea is mainly grown on residual moisture, and yields suffer from poor plant stands. Yield potentials are about 1300-1500 Kg/ha around Hyderabad increasing to 4-5000 Kg/ha in the north of India. Nodulation can be absent or poor, and large responses to inoculation have been obtained when chickpea follows rice. Wilt and root rot diseases, a stunt (Viral?) disease and *Ascochyta* blight can cause serious losses. The pod borer *Helicoverpa armigera*, is the only major insect pest.

The pigeonpea production of 1.72 million t occurs mainly in India (92%) and Africa (4%) as an intercrop with cereals and cotton. The crop is planted in the wet season producing pods on the residual moisture in the dry season. Pigeonpea is susceptible to waterlogging and frost. *Fusarium* wilt is the most serious disease, but pod boring insects are also very damaging. Yields depend on the location, and can be up to 3000 Kg/ha. N uptake continues until maturity. Leaf fall can amount to 2,200 Kg/ha D.M. containing 36 Kg N.

Cowpeas are the major grain legume in Africa, with Nigeria, the major producer. Recorded production is about 1 million t, mostly in the SAT; elsewhere diseases reduce yields and everywhere insect damage can reduce yields to less than 200 Kg/ha. With insect control, sole crop yields in farmers fields can exceed 2t/ha. Cowpeas are usually intercropped with cereals and this reduces yields. Other SAT grain legumes include mung bean, green gram, horsegram, guar, moth bean, and Bambara groundnut.

Most cropping systems in the SAT are based on subsistence farming, with grain legumes invariably included. In India, on black and alluvial soils, legumes can be grown in both kharif and rabi seasons. In red soils cropping is usually in the kharif season only. In Africa, indigenous cropping systems prevail but the periods under bush fallow are shortening. Grain legumes, except for groundnut cashcrops, are usually intercropped with sorghum and millet. Mixed cropping is the norm for many SAT legumes, and can produce higher yields and monetary returns than sole crops grown on the same area.

Legumes in the SAT play an essential role in maintaining soil fertility. The amounts of nitrogen returned to the soil depend on the harvesting procedures. Generally grain legumes do not deplete soil nitrogen, some, like pigeonpea and the tree *Acacia albida*, probably augment soil N through leaf fall. Forage legumes such as *Stylosanthes* spp. can add as much as 93 Kg N/ha/annum to the soil.

Grain legumes play an essential role in the nutrition of people in the SAT, balancing the deficiencies of the basically cereal diet. Calory deficiency seems more acute than protein deficiency. Legumes are a preferred item in the diet, and it is likely that their intake will increase, with yield increases of both cereals and legumes.

CHARACTERISTICS OF THE SEMI-ARID TROPICS

The delineation of the semi arid tropics (SAT) is based on the

classification of Troll (1966), who distinguished five climatic regions within the tropics, with two semi-arid zones (dry & thorne savannah) as follows:

V3: Wet and dry tropical climates with 4.5 to 7 humid months.

V4: Dry tropical climates with 2 to 4.5 humid months.

In humid months, precipitation exceeds or equals potential evapotranspiration. The semi-arid tropics are thus characterised by an arid season lasting from 5 - 10 months. An arid month is one where potential evapotranspiration exceeds precipitation. The annual rainfall in this semi-arid tropical region varies from about 500 to 1500 mm with more than 95% of the annual precipitation falling in the rainy season which generally lasts from 4-7 months. The rainfall is undependable - with the start of the monsoon varying considerably, and extremely variable in amount - both from year to year and between seasons. Brief and/or extended droughts are frequent. Much of the rain occurs in high intensity storms resulting in run off. The soils are often shallow in depth with limited water holding capacity. Although the total rainfall is enough to grow one or two good crops a year, food production is unstable and yields low. Because of the erratic nature of the rains farmers are reluctant to adopt new production techniques - often because they do not have sufficient capital.

The semi-arid tropical zones of the world occur in all or part of the land mass of 48 countries in four continents with an estimated total area of 19.6 million Km^2 and population of 512 million people. About 34% of the total land area of these countries is semi-arid. Australia has the largest SAT area in the world (19% of the total) followed by India (9%). Approximately 260 million Indians (or 51% of the total SAT population) reside in the SAT. Other countries with significant areas or populations in the SAT are shown in Table 1

About half the world's sorghum grain is produced in the SAT countries, and 40% of the millet, 90% of the chickpea, 95% of the pigeonpea and 64% of the groundnuts (Tables 2 and 3).

ICRISAT

In 1972 the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was established with headquarters at Hyderabad, India. ICRISAT is financed by many donor countries and organizations under the aegis of the Consultative Group on International Agricultural Research. The research programme at ICRISAT has four main objectives:

1. Crop Improvement, serving as a world centre for the preservation and improvement of the genetic resources of sorghum, millet, chickpea, pigeonpea and groundnut. Currently the germplasm collection contains 12,466 entries of sorghum, 5,003 of pearl millet, 10,842 of chickpea, 5,530 of pigeonpea and about 2000 lines of groundnut. The groundnut improvement programme started in 1976, and much of the germplasm is still being acquired. Breeders

Pathologists, Entomologists, Physiologists, and Microbiologists are involved in the crop improvement programme.

2. Developing Farming Systems which will help to increase and stabilise agricultural production through better use of the natural and human resources. The approach is two-fold:
   (i) To investigate the natural resources of the various regions of the SAT. To analyse these resources and assist in developing means for improving, managing and utilising these resources more effectively.
   (ii) To develop cropping systems which will make the optimum short and long term use of the natural environment under existing constraints.
3. Identifying the Socio-Economic and other Constraints to agricultural development, and evaluation of alternative means of alleviation them via technological and institutional changes.
4. Assisting National and Regional Research Programmes through cooperation and support, and by sponsoring conferences and operating training programs on an international basis.

The Government of India provided 1360 ha of land for the Institute's headquarters, 25 Km northwest of Hyderabad. This land includes both red and black soils characteristic of vast areas of the SAT.

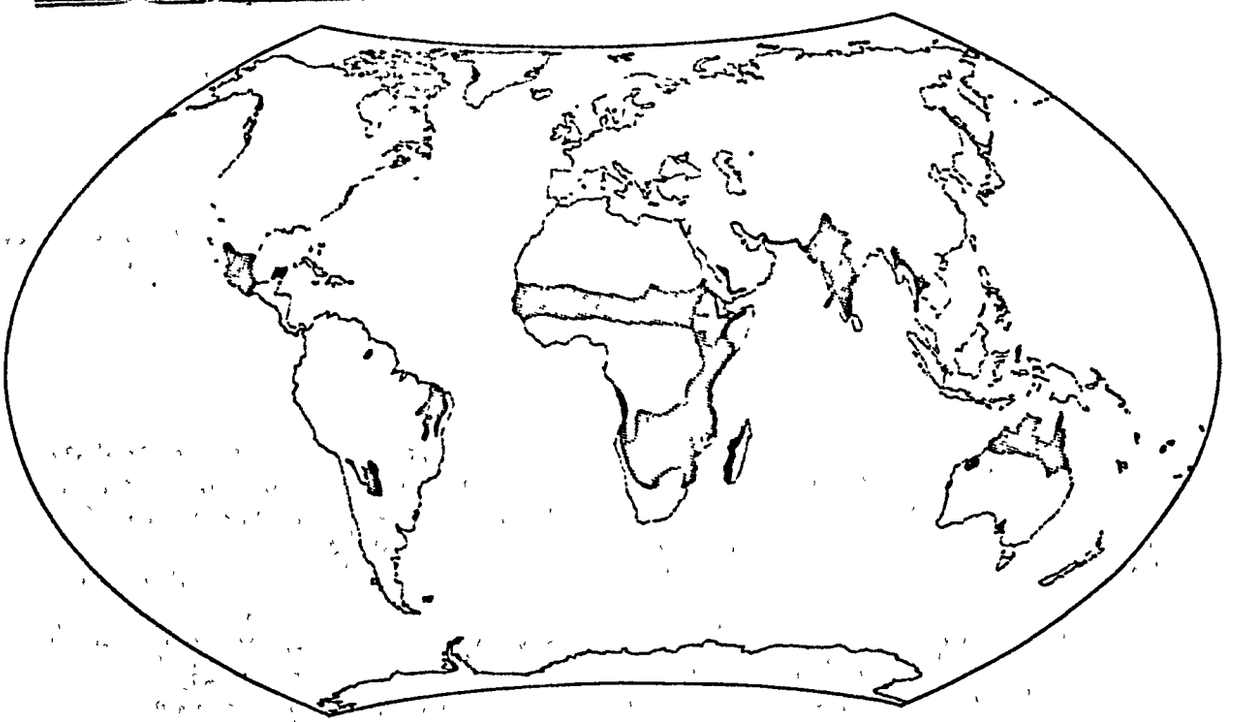

Figure 1. Semi-arid tropical zones in the world. (From Troll, 1966).

Table 1: Estimated Area and Population of some Semi-Arid Tropical Countries

Country	Portion of country Semi-arid (%)	Semi-Arid Area 1000 Km^2	% of total SAT area	SAT Population (million)	% of total SAT population
Australia	25	1921	10	1	0.2
India	54	1700	9	261	51
Sudan	60	1491	8	15	3
Mexico	60	1183	6	29	5
Brazil	12	1036	5	20	4
Nigeria	75	693	4	42	8
Tanzania	60	752	4	10	2
Zambia	100	753	4	4	1
Angola	60	748	4	3	1
Mali	60	721	4	5	1
Niger	40	507	3	4	1
Chad	40	514	3	3	0.5
Botswana	100	569	3	1	0.2
Thailand	60	308	2	25	4

Figures taken from Ryan (1975).

CROP PRODUCTION

Cereals dominate the SAT grain production (Tables 2 & 3), with 43.7 million tonnes per annum of sorghum plus millet produced on 67.6 million ha, compared with 30.8 million tonnes of grain legumes on about 47 million ha (means for 1972-74). Grain legumes include both pulses

Table 2. Area, Production and Yield of Major Crops in the Semi-Arid Tropics.*

	Area 10^3 ha	Production 10^3 t	Yield Kg/ha
Sorghum	34,265	26,354	769
Millet	33,309	17,301	519
Total Cereal	67,574	43,655	635
Groundnut	14,771	10,858	735
Chick pea	9,541	5,770	605
Pigeon pea	2,575	1,684	654
Dry beans and soybeans	20,485	12,469	609
Total grain legumes	47,372	30,781	650

*Values are means for the years 1972, 1973, 1974 except for dry beans and soybeans which are for 1970, 1971, 1972. Source: FAO Production Year Books, data compiled by J.G. Ryan & M. Asokan, ICRISAT.

Table 3. Semi-Arid Tropical Production of Food Legumes (1971) (1000 tonnes)

Region of Semi-Arid Tropics	Dry Beans	Soy-beans	Chick-peas	Cow-peas	Pigeon peas	Ground-nuts
Asia	2265	1228	5770	10	1910	6977
Africa	613	65	205	1069	51	3847
Central and South America	2641*	2227	173	1800*	26	1459
TOTAL: Semi-Arid Tropics	5319	3520	5948	2079	1987	12242
TOTAL WORLD	10273	48457	6594	1995	2024	18211

Source: FAO Production Yearbook 1972, Rome Italy. (Values based on total production for countries with SAT regions.)
Estimated: Dry bean production listed in FAO yearbook for Brazil includes cowpea and *Phaseolus vulgaris*.

and oil seeds; pulses are edible legume grains other than the oil seed grains, soybean and groundnut. In Africa, and Central and South America, SAT maize production is also significant. Wheat is also grown in the cool (rabi) season in SAT India and the advent of increased irrigation facilities, new high yielding wheat varieties, and increased fertilizer usage has affected pulse production. More area has been sown to wheat, and this has partly been at the expense of chickpea, pigeonpea and other pulses. In India, the total area under pulses increased by 9% or 1.96 million ha from 1954-55 to 1964-65 before the "green revolution" in wheat began. Production increased by 1.47 million tonnes or 13%. In the same period the area under wheat increased by 2.16 million ha, or 19%, while production increased by 3.21 million tonnes, or 36%.

In the subsequent nine years (to 1973-74), the area sown to wheat increased by 5.64 million ha (42%) with an increase in production of 80% or 9.82 million tonnes. The area under pulses production fell by 4.2% and production by 21.4% during this period, the relatively greater decrease in production suggesting that it was the better land which changed to wheat production. Table 4 shows these changes in production in the six major wheat producing states of India, calculated from trend lines fitted to the production records from 1964-65 to 1973-74 (J.G. Ryan & M. Asokan, ICRISAT MS in preparation). The largest reduction in area occurred for chickpea amounting to 15.4% or 1.154,000 ha. pigeonpea and other pulses areas also declined.

Table 4. Net Changes in Trend Areas of Wheat and Pulses in Six Major Wheat growing States of India, 1964-65 to 1973-74*

Crop	Area of Crops from Trend Line in ($x10^3$ ha)			Difference as Percentage of 1964 - 65 (%)
	1964-65	1973-74	Difference	
Wheat	10,242	17,319	+ 7,077	+ 69.1
Chickpea	7,505	6,351	- 1,154	- 15.4
Pigeonpea	1,322	1,183	- 139	- 10.5
Other pulses	6,597	6,385	- 212	- 3.2
Total pulses	15,424	13,919	- 1,505	- 9.8

*J.G. Ryan and M. Asokan, ICRISAT, MS in preparation.

Trend lines from 1964-65 to 1973-74 drawn from data in Directorate of Economics and Statistics, India.

However, only 21% of the increased area of wheat could be accounted for by the decline in area under pulses, with increases in cropping intensity and production on previously uncropped land accounting for most of the wheat increase. Giving the 1961-62 production and area sown a value of 100, by 1974-75, total cereal production in India had increased to an index value of 131 but area to only 108 while total pulse production fell to a value of 86 with an area index of 93. Thus much of the increased cereal production came from higher yields, and part of the decline in pulse production can be attributed to lower yields. Groundnut production fluctuated considerably mainly due to yield variations but had increased to an index value of 108 in 1974-75 (Indian Agriculture in Brief, 1976). At the same time there was a general shift in the areas under pulse production from the North of India into the Northern Central states (M. von Oppen, ICRISAT, pers. comm.).

Although wheat and rice yields have recently increased considerably in India, yields of the major grain crops grown in the semi-arid tropics changed little during the period from 1958-61 to 1972-75. Sorghum yields increased from 507 to 547 Kg/ha, and millet yields from 317 to 397 Kg/ha, while chickpea yields decreased by 9% from 636 to 580 Kg/ha with a smaller decrease for pigeon pea and groundnut to 677 and 709 Kg/ha (Table 5). Despite the low yields of the grain legumes, they are still higher than that of the coarse cereals in SAT India. Yields for all these crops can be almost doubled merely by improved management. Further increases beyond this are being obtained with the most recent varieties developed. The low yields of SAT crops reflects not only the unfavourable environment and low availability of inputs, but also the small amount of research devoted to date to improving these crops.

Farm sizes in the SAT regions of the less developed countries are small. In SAT India 90% of the holdings have an area less than 6 ha and such holdings constitute 48% of the total SAT land area. Thirty-nine percent of holdings are less than 0.4 ha and 54% less than 1 ha in size. For SAT Asia as a whole the average agricultural holding is 2.72 ha (Ryan, 1975).

In Semi-arid Nigeria the average farm size is 1.59 ha, while 81% of the farms are less than 4.1 ha in size (Norman, 1976).

Fertilizer use in the SAT countries is small, with less than 16 Kg/ha of N P K applied annually in Asia, 3.12 Kg/ha in Africa, and 30 Kg/ha in South and Central America (Ryan, 1975). Fertilizer use on just the SAT regions of these countries is even less. For India almost half the SAT farms have some irrigated land, mostly used for producing rice, wheat, sugar cane etc. and not for pulses. Any fertilizer that is used on SAT farms is almost certainly used on such land. In a survey of unirrigated farms in India in 1970-71, only 2.67% of the area under pulses was fertilized, receiving 9 Kg N and 3.6 Kg P_2O_5/ha/annum. Only 13% of the farmers applied fertilizer to irrigated land under pulse production and this was to only 15% of the gross area under irrigation. A much higher proportion of other irrigated crops receives fertilizer (Fertilizer Use on Selected Crops in India, 1974).

Table 5. Area, Production and Yield of Grain Crops in Semi-Arid Tropics in India*.

Crop	1958-61			1972-75		
	Area $x10^3$ ha	Production $x10^3$ t	Yield Kg/ha	Area $x10^3$ ha	Production $x10^3$ t	Yield Kg/ha
Sorghum	18,237	9.209	507	16,028	8,762	547
Millet	11,197	3,548	317	12,337	4,893	397
Groundnut	6,385	4,850	760	7,060	5.005	709
Chickpea	9,894	6,297	636	7,293	4,231	580
Pigeon pea	2,617	1,847	746	2,537	1,718	677

*Data Figures compiled by M. von Oppen, ICRISAT, from data in Directorate of Economics and Statistics, India.

GRAIN LEGUME PRODUCTION

In most of the semi-arid tropics, grain legumes are about the only legumes sown by farmers. Some pasture legumes are sown in Australia and in irrigated regions, lucerne and berseem clover are sown in the cool (dry) season in India. The production of the major grain legumes involved is shown in Table 3. Most production is of groundnuts and dry beans (mainly *Phaseolus vulgaris, P. lunatus, Vigna radiata, V. mungo, Dolichos bifloris)* followed by chickpea, soybean, cowpeas and pigeon peas. Much of the soybean production in South America is in areas outside the semi-arid zone.

Groundnut

The cultivated groundnut, *Arachis hypogaea* L. is native to south America with a primary gene centre probably on the eastern slope of the Andes in Bolivia (Krapovickas, 1968). There are two subspecies distinguished on the basis of branching pattern - *fastigiata* with a sequential system and *hypogaea* with an alternate system.

The value of groundnuts as a food, as a base for a non-alcoholic drink, and in making soap has been long recognised by the indigenous inhabitants of South America. Its use was well established from Brazil to Bolivia by the time the Spaniards and Portuguese arrived. From there it spread to Peru, Mexico, China, Java, Africa, India, USA by the beginning of the 19th century (Peanuts- Culture and Uses, 1973). It is grown in 82 countries on 19 million ha, with most production in Asia (10.0 million tonnes), Africa (4.46 million tonnes), and

North America (1.74 million tonnes). Two thirds of the total world production is in the semi-arid tropics. India is the largest producer of groundnuts in the world with about 5.0 million tonnes produced on 7 million ha at an average yield of 709 Kg/ha over the period 1972-75 (Table 5). Most of the Indian production (93%) is in the Kharif or monsoon season with the rest in the rabi season usually as an irrigated crop. In Africa, Nigeria and Senegal are the major producers, each with about 17% of the African production. Average yields in Senegal at 760 Kg/ha are much higher than in Nigeria at 438 Kg/ha. Yields and production are given as nuts in shell for groundnut, with an average shelling percentage of 70%.

The groundnut kernel contains up to 50% of a non-drying oil (twice that of soybean), which is easily extracted, and, with little processing, used for cooking and making soap and margarine. The residual cake after pressing out the oil is used for animal feed. Groundnut is also an important food crop and a good supply of thiamine, with up to 25% readily digestible protein. The haulm left after harvesting is valuable animal feed.

The main exporting countries are Nigeria (35% of its production making up 27% of the total world trade); Senegal (65% of its production and 18% of the trade); India (13% of the trade); and Argentina, (5% of the trade).

Although the world average yields of groundnut are low (884 Kg/ha, average for 1972-74) the yield potential approaches that of the best cereal yields. In the USA farmers in some areas average 3000 Kg/ha nuts in shell, reaching 6,500 Kg/ha in parts of Florida. In Rhodesia 9000 Kg/ha has been obtained with disease control and supplemental irrigation, and in Malawi, 7000 Kg/ha on a research station with disease control but no irrigation (Gibbons, 1976).

Groundnuts are usually grown as a sole crop, but in Africa mixed cropping with cereals is also practiced. Yields are usually depressed in mixed cropping to around 300 Kg/ha. In farmer's fields in Northern Nigeria, yields were increased 43% to 485 Kg/ha by addition of 15 Kg P_2O_5/ha as superphosphate (Norman, Bruntjer, and Goddard 1970). Groundnuts are relatively day length insensitive so that cultivars can be evaluated over any latitude. Groundnut yields more under low inputs than soybean, cowpea or *Phaseolus vulgaris*, and the crop is well adapted to the semi-arid tropics.

The main disease problem is *Cercospora* leaf spot (*C. arachidicola*, early leafspot and *C. personatum*, late leafspot) and losses vary from 15-50%, estimated to be 3 million tonnes per year worldwide. No worthwhile resistance has been found in cultivated groundnuts, although two wild *Arachis* spp which can be crossed with *A. hypogaea* have high resistance (Abdou, 1974).

Ruse caused by *Puccinia arachidis* has recently spread from South America to all major producing areas. Very heavy yield losses occur, particularly in conjunction with *Cercospora* infection. Rust resistant breeders lines are reported to be available. *Aspergillus flavus* infection of groundnuts can occur throughout the world,

and in certain conditions this produces a very potent aflotoxin, usually in the post harvest period, which causes liver damage and is also carcinogenic. The presence of aflotoxin stops trade, and a very large amount of research has been directed to preventing its formation, particularly by post harvest techniques. Resistance to invasion by the fungus has been found for two breeding lines if the testa of the nut remains intact (Gibbons, 1976).

Insect pests tend to be location specific, but there are some reports of resistance or non-preference feeding (antibiosis) or tolerance to armyworm, thrips, southern corn rootworm, parasitic nematodes and mites. Termites can be a major problem at all stages of plant growth.

Generally groundnuts seem to be well nodulated. Twenty-one percent increase in yield was obtained with a granular *Rhizobium* inoculant in Florida in newly cleared fields (Cobb & Whitty, 1973). In other countries yield responses to nitrogen fertilizer have been reported suggesting that nodulation may be poor (Peanuts, Culture & Uses, 1973). Nodulation by native rhizobia seems to be effective in Nigeria, Senegal and India, although maggots eat some nodules in red soils at ICRISAT. Groundnut nodulates freely with a wide range of *Rhizobium* strains (Dart, 1974). The yields quoted earlier indicate that groundnut nodules can fix a great deal of nitrogen, estimated to be up to 240 Kg N/ha or 80% of the plant's nitrogen uptake in experiments in northern Nigeria. The yield of 7000 Kg/ha nuts in shell in Malawi suggests a total nitrogen uptake by the plant of 334 Kg N/ha. With a shelling percentage of 70% and nut N content of 4%, the nuts contained 196 Kg N and the shells at 1% N contain 21 Kg N. Nuts in shell contain about 65% of the total plant nitrogen; haulm N content at harvest is about 2%. If we assume a soil mineral nitrogen availability of 70 Kg N/ha/season the N fixed by this crop would be at least 260 Kg N.

Calcium deficiency in the soil severely restricts pod filling and yields. Sulphur deficiency can also be a major factor limiting yields of fertilised groundnut in Northern Nigeria (Bromfield, 1975) and elsewhere (Peanuts - Culture and Uses, 1973). The crop responds well to phosphorus and sulphur additions, even in the form of Togolese rock phosphate mixed with about 10% elemental S (Bromfield, 1975). Responses to molybdenum sprays have also been obtained in Northern Nigeria (Heathcote, pers. comm.; Bromfield, 1975).

Chickpea

Chickpea *(Cicer arietenum* L.) has been long cultivated - with specimens found at Hacilar in Turkey dating back to about 5,400 years BC (Helback, 1970); Homer referred to chickpea in the Iliad (1000-800 BC). The crop apparently originated in Western Asia, with the wild annual *Cicer reticulatem,* now found in South East Turkey the probable progenitor (Auckland and Singh, 1976). Chickpea is now cultivated in over 31 countries, from Mexico and the USA through Spain and Mediterranean countries, Morocco and North African countries, to West Asia, and to India (Van der Maesen, 1972). Two main types are cultivated:

(1) the large seeded, ramshead-shaped or round seeded, chickpeas with more than 26 g per 100 seed, and creamy colour. Plants are medium to tall with large leaflets no anthocyanin and white flowers, and are characteristic of the Mediterranean region, known on the Indian sub-continent as the Kabuli type; and (2) small seeded types; irregularly shaped and variously coloured, plants small with small leaflets (6-9 mm), sometimes prostrate and mostly with anthocyanin and purplish pink flowers. These are characteristic of East Asia, Ethiopia, parts of Iran and Afghanistan, known as Desi (meaning indigenous) types.

Chickpeas can also be grouped into winter (October/November) plantings, mainly of Desi type - from Pakistan eastward, but also in Ethiopia, Sudan, Mexico and Chile; and summer planting, mainly Kabuli type, from Afghanistan westwards into the Middle East, Southern Europe and North Africa. Desi types adapted to summer plantings overlap in Iran and Afghanistan. Flowering can start from 28 days after planting, but large variation exists between lines in time to flowering, in flowering duration and in days to maturity. These durations are much affected by the environment and location. Chickpea is self pollinated. Flowering habit is indeterminate and flowering is hastened by long days, with large differences in this response between cultivars (e.g. Dart, Islam, Eaglesham, 1975).

The Kabuli and Desi types have probably been separated for thousands of years. Some Kabuli types were introduced into the Indian sub continent about 1700, but are poorly adapted as yet.

Chickpeas are grown on about 9.5 million ha (Table 2) with a production of about 5.8 million tonnes and average yield around 600 Kh/ha (Tables 2,3). Ninety percent of the total production is in the semi-arid tropics. It is the most important pulse crop in India, with 74% of the worlds production, followed by 10% in Pakistan and 4% in Ethiopia. Other countries, in decreasing order of production, are Mexico, Burma, Spain, Morocco, Turkey, Iran, and Tanzania (see Singh and Auckland, 1975, which also lists yields). An increasing demand in Arab countries is stimulating exports mainly from Ethiopia and Sudan. Mexico supplies European and South American countries, but export trade is small.

Chickpeas are eaten raw or roasted, but are usually consumed after boiling. In India, Pakistan and Bangladesh this is usually in the form of 'dhal', prepared by splitting the seeds, separating them from the testa, and boiling with spices. Chickpea flour is used in many Indian confectionaries, or in biscuits (in Ethiopia). In Arabian countries 'hommos' are prepared, and in Ethiopia chickpeas are boiled, ground and mixed with spices to make 'Shero Wat'.

Average yields in India are currently about 580 Kg/ha (Table 5), and have declined by 9% over the last 14 years. This may partly be the result of a change in the location of production, associated with increased cereal production in some North Indian districts (see Section 3). The increase in area of production of chickpea in some states during this period has been associated with a decrease in yield (e.g. in Madhya Pradesh from 587 to 529 Kg/ha). Chickpeas in some farmer's fields in

this state are not nodulated and in other areas adjacent plants vary a great deal in their nodulation (author's observations), suggesting that the appropriate rhizobia may be lacking or sparse in the soil when chickpeas are introduced as a new crop. Responses to inoculation have been obtained (Sahni, These Proceedings; Subba Rao, 1976). When chickpea follows rice, yields are often poor; large responses to inoculation were obtained in a black soil at Hyderabad with a 134% yield increase over the uninoculated control, to give 1907 Kg/ha (Srirama Raju and Samuel, 1976).

Chickpeas are grown on a variety of soils from heavy-black to silt loams and loess soils, to more sandy soils, e.g. parts of Rajasthan, usually with neutral to alkaline pH. The crop is quite susceptible to saline soil conditions and Kabuli types to iron deficiency, producing a leaf chlorosis. Chickpeas are usually grown as a rainfed crop on residual moisture although responses to irrigation can be obtained. This needs to be carefully controlled and a single irrigation during pod fill is often beneficial (Saxena and Yadav, 1975). The response to phosphorus fertilizing has been variable, possibly depending on the soil P status, but this may be related to the location of the added phosphorus in the upper soil layers which usually become progressively drier during the season. The main cause of low yields is probably the poor plant stands, resulting from poor seed bed preparation and a low germination percentage.

The only major insect pest is *Helicoverpa (Heliothus) armigera*. (Davies & Lateef, 1975). Active nodules can be eaten by grubs (author's observation). Wilt caused by *Fusarium oxysporum*, root rot by *Rhizoctonia bataticola*, and a stunt disease (viral?) transmitted by *Aphis craccivora* are widespread in India and can reduce yields considerably. *Ascochyta* blight can also be serious under some environmental conditions (Nene and associates, 1976).

High soil temperatures affect chickpea nodulation - with soil temperatures above 30°C preventing nodulation, and daily cycles where the temperature rises to 33° reducing nitrogen fixation by already formed nodules. *Rhizobium* strains can be selected which fix more nitrogen at 30° soil temperature (Islam, 1975; Dart, Islam & Eaglesham, 1975). At Hyderabad nitrogen uptake by chickpeas reached a maximum soon after flowering and then declined, largely because of leaf fall. After 50-60 days, the percentage nitrogen in leaves and stems declined, with nitrogen remobilised from stems and leaves accounting for 48-96% of the nitrogen in the grain, depending on the cultivar. Yields appear to be limited by source supply at Hyderabad, around 1200-1500 Kg/ha in large experimental plots. In the north of India, yields are much greater and in the 1975-76 season at Hissar were 4-5000 Kg/ha in similar experiments (Saxena & Sheldrake, 1976). Breeders material is giving even higher yields. A yield of 4000 Kg/ha (seed protein of 18%) contains 115 Kg N. With a harvest index of 44% and haulm N content of 1.25%, the rest of the tops contain about 64 Kg N. Roots and nodules, with about 1.5% N content, and with an estimated toproot ratio at harvest of 6, contain about 13 Kg N.

Total plant N uptake at harvest is about 192 Kg N/ha, giving an estimated nitrogen fixation of at least 120-140 Kg N/ha.

Around Hyderabad (Latitude 17°N) maturity occurs in about 110 days after planting, while at Hissar (Latitude 29.5°N) average maturity time is around 165 days. The curtailment of the growth period at Hyderabad seems to be largely related to the soil moisture deficit which develops in the top 30 cm soil. Although roots penetrate to much lower depths (up to 120 cm for the one cultivar examined) over 95% of the nodules occur in the top 15 cm soil, although during pod fill 75% of the roots occur below 30 cm in the soil. Most nodules are formed early in plant development, and have almost completely senesced as pods start to fill. By this stage they are also in dry soil. Nodules on irrigated plants remain active for much longer. In the 1975-76 season at Hissar, nodules remained active and vegetation green until a fortnight before harvesting, so that nodule senescence is not necessarily linked to pod fill. Moisture was not limiting at Hissar, and final senescence was probably initiated by rapidly rising temperature.

In the ICRISAT breeding program large yield increases have been obtained by crossing Kabuli x Desi types, and for East Asia backcrossing these with Desi types, and for West Asia, with Kabuli types. Further details of the breeding methodology are given by Auckland & Singh (1976). The yield potential for chickpeas is very high, up to 6 times that presently obtained by farmers.

Pigeonpea

Cajanus cajan (L) Millsp., commonly known as pigeonpea, red gram, tur, arhar, gandul etc, ranks 6th in total world production of pulse legumes - with 1.720 million tonnes or 3.9% of the world total pulse production in 1972. Apart from the reported production, pigeonpeas are grown on bunds, as hedges and trees, adding to the farmers own food production. The plant type varies a great deal depending on the locality and duration of the cultivar. The crop is seldom sown in pure stands, so production figures may be a gross underestimate. India accounts for 92% of the recorded production (1.57 million tonnes) followed by 4% in Africa (Uganda, Tanzania, Malawi and Kenya), and 1.7% in Burma.

Pigeonpeas are grown in most tropical countries although production may be small. The average yield level since 1908 in India has been around 600-700 Kg/ha. The grain is mainly consumed as dhal, although green peas are used in some countries and in others the crop is used for forage.

Pigeonpea has a remarkable ability to utilize residual moisture during the dry season, hence its value as a crop for semi-arid regions. In India the crop is grown in areas with 500 to 1500 mm rainfall, on a wide range of soil types - from light, shallow, red soils to heavy, black clay soils. However the crop is susceptible to waterlogging although some lines are more tolerant (Green and Associates, 1976). Pigeonpea grows well on alkaline and saline soils and also seems adapted to soil pH's as low as 5. Frost kills pigeonpeas so that the crop is mostly grown in frost free areas, or early maturing varieties are sown.

Farmers usually sow late maturing varieties as a companion crop, usually with sorghum, pearl millet, cotton or other, short duration legumes and minor millets. Crop production seems to be little affected by fertilizer and water management (Saxena and Yadav, 1975). The objectives of the breeding programme at ICRISAT are to replace existing types with high yielding cultivars having stability of yield through resistance to disease and insect pests or compatibility with insect control measures (Sharma and Green, 1975). Cross pollination, largely by bees, occurs to a limited extent.

The most serious disease is *Fusarium* wilt, found in almost all countries where the crop is grown. Sterility mosaic virus also causes serious damage in India. Genetic sources of resistance to wilt in certain localities are reported, and five germplasm accessions and a released variety (HY-3C) are resistant to sterility mosaic virus (Nene and associates, 1976).

Pod boring insects - 12 in number - are the most universal limitation on yield with *Helicoverpa (Heliothus)* probably the major pest, and as yet there is no identified resistance. "Determinate" types in which flowering begins on the topmost branches which may be easier to spray also seem more susceptible to lepidopterous borers in unsprayed conditions (Davies and Lateef, 1975).

Yields vary a great deal with the plant protection afforded, with plant duration and with location. Pigeonpea is photosensitive and date of planting has a great influence on morphological development, time to maturity and yield. Medium variety cultivara at Hyderabad maturing in 160-180 days yield up to 2440 Kg/ha grain on red soil with corresponding yields of 1700-1800 Kg/ha in black soil with insecticides sprays, while longer season varieties in Northern states of India yield up to 3000 Kg/ha. After ratooning some medium varieties produce a second crop during the summer, with as much as 40% of the total yield, with the deep rooting system making maximum usage of the residual soil moisture. The woody parts of the plant are useful as fuel.

Traditionally pigeonpea is rarely sown with its companion crop in alternate rows - often one row of pigeonpea is separated by 4-10 rows. Studies are in progress at ICRISAT on the effect of mixed cropping patterns on pest incidence, particularly pigeonpea pod borers and sorghum shootfly. Some varieties develop differently in intercropping situations, where the intercrop shades the pigeon pea. For example basal branches did not develop on the varieties HY3-C and ICRISAT 1 when grown in alternate rows with pearl millet. This suggested that varieties should be screened in appropriate intercrop situations if they are to be used as an intercrop.

Yield seems well correlated with pod number and vegetative growth (plant height, number and length of branches). Defoliation experiments suggest that assimilates are not limiting yield. Seed number per pod and 100 seed weight are quite constant regardless of where the pods are borne on the plant. In long duration varieties, 50% defoliation at flowering had no effect on yields. In the determinate variety ST-1, removal of all flowers over a 6 week period reduced yield by

only 22%. Pigeonpea has a remarkable adaptation to flower drop. Interestingly, wilt was prevented in an off season planting by flower removal suggesting that pods reduce the amount of photosynthate going to the roots and this allows penetration, or expression, of the wilt fungus (Narayanan and Sheldrake, 1976).

Whether nodules are also starved for photosynthate during pod-fill remains to be seen. Active nodules are present during early pod fill - and as yields do not seem limited by photo-assimilate supply, perhaps nitrogen supply is also non limiting. Nitrogen uptake by pigeonpea continues until maturity for both early and medium maturity varieties. There is only a slight decrease in the amount of N in leaves as reproductive structures form. Stem nitrogen contents slightly increased during this period. Leaf fall can amount to 2,200 Kg/ha of debris in medium duration cultivars in monoculture and at an average 1.63% N, amount to a return of 36 Kg N/ha. This could have a significant influence on soil nitrogen levels.

Inoculation experiments have only occasionally given increases in yield (e.g. Subba Rao, 1975). Preliminary observations of pigeon peas sown after rice at ICRISAT, show an enhancement of nodulation due to inoculation. Nodule development on young plants in red and black soil is quite different. More nodules form and remain active in the lighter red soil, with up to 80% of the nodules in black soil senescent by 30 days. Part of this senescence is due to attack by a grub eating the cortex of the nodule leaving a hollow shell. Nodules continue to form throughout plant growth and may be found 75 cm in a horizontal direction from the stem base (M.R. Rao, ICRISAT, unpublished observations, 1976). Nodules also occur at depth through black soil - and could be found in the 120-150 cm deep zone, and sometimes even below 150 cm. Although most nodules occur in the top 30 cm, up to 35% of nodules occured at lower depths (Narayanan and Sheldrake, 1976).

Cowpeas

Cowpea *(Vigna unguiculata* (l.) Walp.) is the most important grain legume in the dry, semi-arid zone of Africa, although it is also grown to a more limited extent in the humid tropics. Africa produced about 95% of the world production of 0.97 million tonnes in 1973, of which Nigeria produced 61%, Niger 13% Upper Volta 5.5%. Most of this is produced as an intercrop in the SAT zone (Summerfield, Huxley & Steele, 1974). As much as 10-15% of production or 170,000 tonnes may be in kitchen gardens, and the production in South America, mainly in North East Brazil, is reported in the FAO statistics as dry beans *(Phaseolus vulgaris)* and may be as much as 2.6 million tonnes per annum (see Table 3; Rachie, 1975). Thus total production could amount to 4.0-4.8 million tonnes per annum.

Steele (1972) suggests that the cultivated cowpea was domesticated in Ethiopia along with sorghum, spreading to West Africa 3-4,000 years ago. Although cowpeas are cleistogamous inbreeders, great diversity has accumulated in West African cultivars (outcrossing from bee activity can be as high as 10%). Many wild and weedy types exist alongside the

cultivated cowpea. The world germplasm collection at the International Institute for Tropical Agriculture, (IITA) Ibadan, Nigeria contains more than 6,000 accessions (Cowpea Germplasm Catalog, IITA, 1974). The plant type varies from the spreading short-day forms usually interplanted with sorghum and millet, to more determinate, upright day-neutral types, interplanted with yams, cassava & maize or grown as sole crops in the more humid zones. Prostrate and climbing types also occur. Cowpeas are eaten as green and dry beans, and the leaves sometimes as a vegetable. The haulms after harvest, or if pods are not set, are good fodder; in Australia cowpea crops are sown for forage. Crop maturity takes from less than 60 days up to 7-8 months, depending on the environment and genotype. Cowpeas are grown on a wide variety of soil types - from sands to heavy clay soils. Most cultivars do not tolerate waterlogging, and after only 4 days nodules rapidly senesce. A small amount of nitrogenase activity persists with continued water-logging as nodule structure adapts (Minchin and Summerfield, 1976).

Insects are the main scourge of cowpea production, with leaf-feeding beetles, leaf-sucking insects, pod sucking bugs and pod borers, and bruchids, attacking the crop from just after sowing to final maturity, causing an estimated loss of potential yield of more than 90% at Samaru, Northern Nigeria (Singh, 1973; Raheja, 1973). Nematodes, particularly three species of rootknot nematode, can cause large yield reductions, estimated to be as high as 20-30% in Nigeria (Caveness, 1973). Resistant or tolerant cowpea lines to some insects and nematodes have been identified (Rachie & Roberts, 1974).

Plant diseases also attack cowpeas in the field from germination to maturity, particularly in more humid conditions. *Rhizoctonia solani* and *Pythium aphanidermatum* can produce seedling mortality of up to 75%. This can be controlled by thiram or chloroneb seed dressing. Anthracnose caused by *Colleototrichum lindemuthianum* on stems, petioles, peduncles pods and leaf veins can reduce yields by 50%. *Cercospora* leaf spots likewise reduce yields by up to 43%, and *Xanthomonas vignicola* bacterial pustule can cause severe leaf spotting, defoliation and yield reduction. Several viral symptoms occur and cowpea mosaic virus can reduce yields by 40-60% if seedlings are infected. Multiple disease resistance in many varieties has been demonstrated (Williams, 1973; Gilmer, Whitney & Williams, 1973).

Yields measured in farmers' fields in Northern Nigeria of unsprayed, sole crops ranged from 100 to 900 Kg/ha (Steele, 1972) and most were less than 300 Kg/ha. By sowing an improved variety, and spraying with insecticides (DDT & BHC) after flowering, yields in 10 farmers' fields were increased to 843-2,696 Kg/ha with an average of 1534 Kg/ha (Raheja & Hays, 1975). The average farmers' yields when cowpea is intercropped with cereals are estimated to be about 160 Kg/ha. Plant populations are low at 10-20,000 plants/ha. Experiments with sole crop cowpeas irrigated in the dry season produced yields up to 4,000 Kg/ha (Steele, 1972) and with insect control in the more humid tropics, yields of 2t/ha can be readily obtained in a 75-85 day growing period (Rachie, 1975).

Shading from the maize intercrop (Wien & Nangju, 1976), and high soil temperatures (Dart & Mercer, 1965; Minchin, Huxley & Summerfield, 1976; Eaglesham & Dart, 1975, 1976) can reduce nodulation, nitrogen fixation and mineral nitrogen uptake and yields. Breeding or selection of cultivars more adapted to shading has not been attempted, but varieties differ in their tolerance of high soil temperature. Cowpea plants supplied up to 240ppm N continuously, or at different stages of the life cycle, do not yield significantly more in pot experiments than well nodulated cowpeas supplied 25ppm N continually in the culture solution. Vegetative development was greatest with combined nitrogen additions. The grain yields per plant were large - 80-100 g/plant (Summerfield *et al.*,1975, 1976, 1977).

Production of Other Pulses

Other SAT grain legumes are mung bean, green gram, horse gram, guar (cluster bean), moth bean, Bambara groundnut, Kersting's groundnut, Tepary bean, hyacinth bean, and locust bean (Kachroo 1970, Rachie & Roberts, 1974).

Mung bean or green gram is now considered to be *Vigna radiata* (L.) Wilczek var *aureus* (formerly *Phaseolus aureus)*, and black gram or urad is now *V. radiata* var *mungo* (formerly *P. mungo)*. The wild ancestors are thought to occur in India. These are important crops in south east Asia, particularly India, which produces about 0.3 million tonnes of green gram and 0.44 million tonnes of black gram on about 2.9 million ha (Kachroo, 1970; Rachie & Roberts 1974). Much of this production is in the SAT zone in the kharif season, or as a relay crop after rice on residual moisture. They are an excellent food with high digestibility and freedom from flatulence. As well as being made into dhal, they are widely used as bean sprouts or young etiolated seedlings.

Black gram seems well adapted to clayey soils such as the black cotton soils in India, although both grams perform better on loamy soils; both seem tolerant of saline conditions. They are excellent cover and green manure crops. Grain yields are from 4-500 Kg/ha although over 2000 Kg/ha has been reported. Maturity is reached in 80-120 days (Rachie & Roberts, 1974; Moomaw, 1976, These Proceedings).

Horse gram *(Dolichos biflorus* L.) is the most widely grown pulse crop in South India, with an estimated production of 390,000 tonnes on 1.8 mi-lion ha. It is grown as a kharif crop and also on residual moisture in the rabi season, maturing in 4-5 months, producing 150-300 Kg/ha dry seed. Horse gram grows to 30-45 cm high, and is well suited to sandy and shallow soils because of its drought resistance.

Guar *(Cyamopsis tetragonolobus* (L.) Taub) has been grown in India for its green pods and for forage since ancient times. It matures in 3.5-5 months depending on the season (summer of kharif), but green pods can be taken in 2 months. Grain yields are usually about 300 Kg/ha but can be much higher, and green forage yields may be as much as 20,000 Kg/ha. Nitrogen fixation and soil nitrogen gains from guar harvested for seed only can be as much as 93 Kg/ha/annum (Wetselaar, 1967).

Moth bean *(Vigna acontifolia*, Marechal) is a short (15-30 cm high)

annual plant with spreading habit grown mainly in India, Pakistan and Burma. Most production is in the Rajasthan state of India where it is generally grown mixed with sorghum, millet or cotton on light sandy soils in the kharif season. It is reputed to be the most drought resistant of the kharif pulses. Seed yields average 200-500 Kg/ha but can be as much as 1500 Kg/ha as a sole crop; dry hay yields can also be high.

Bambara groundnut *(Voandzeia subterranea* (L.) Thou) is most extensively cultivated in Africa with Nigeria (100,000 tonnes), Niger (30,000 tonnes) and Ghana (20,000 tonnes) estimated to be the main producers although it is also grown in East Africa. The crop grows on very poor, sandy soils which are marginal for other pulses, as a mixed crop with millet or sorghum, or in pure stands, maturing in about 4 months. The fruits are developed in the soil, which is often earthed up to help the process. Diseases and pests are few and dry seed yields are around 500 Kg/ha but much higher yields of shelled nuts have been reported. Kerstings groundnut *(Kerstingiella geocarpa* Harrus) superficially resembles Bambarra groundnut although its plant habit is botanically different. The crop is important in Dahomey, Upper Volta and Sudan.

Tepary bean *(Phaseolus acutifolius* Gray var. *latifolius* Freem) probably originated in south western United States and Northern Mexico where it occurs wild and is well suited to hot, low humidity conditions and can mature in two months if moisture is limiting. Rainfed yields of 500-700 Kg/ha dry seeds can be obtained. It is grown to a limited extent in Southern Asia and Africa.

Hyacinth bean *(Lablab niger* Medik) or lab lab is used as a green bean vegetable or the dry seeds as a pulse. It is also grown as a forage and cover crop. The crop is a hardy, herbaceous, perennial herb usually grown as an annual and is often twining. It is often grown around the household; in the field it may be intersown with cereals such as ragi *(Eleusine coracana)*. Grown in India and west Africa.

Locust bean *(Parkia* spp.) are large trees important in the African savanna, but are seldom planted. The seeds are not a staple food but are cooked and fermented to make a condiment for food flavouring. The fruit pulp from immature pods is used to sweeten drinks and desserts. The trees with their deep roots build up soil fertility in their environs.

CROP MANAGEMENT SYSTEMS

Most cropping patterns in the semi-arid tropics are based on traditional systems of subsistence farming where every farmer tries to produce his own needs. Grain legumes, even though poorly productive, usually occupy some niche. In the semi-arid tropics grain legumes and cereals are often grown together and often with other plants such as cassava - either intercropped or in other mixed cropping patterns. Since the length of the available growing season ranges from about 80 to 200 days, double cropping is generally not feasible on the lighter

Table 6. Cropping Pattern in Low Rainfall-Unirrigated Areas of India*

	Area under the crop as % of total cropped area		Proportion of All-India acreage in low rainfall areas (%)
	In low Rainfall areas	In All-India	
Sorghum	22.92	11.92	64
Millet	11.61	7.56	51
Wheat	7.66	8.36	31
Ragi	2.35	1.69	46
Maize	1.62	2.93	19
Barley	1.40	2.20	21
Chickpea	6.27	6.66	32
Pigeon pea	2.33	1.65	47
Groundnut	9.17	4.15	74
Other oilseeds	3.31	3/30	37
Cotton	9.55	5.28	61
Other crops	21.81	44.40	-
TOTAL	100.00	1.00.00	

*Average of 84 districts. Low rainfall areas, 400-1000 mm annually. Taken from 'A New Technology for Dryland Farming', Indian Agricultural Research Institute, New Delhi, 1970.

soils with low moisture holding capacity. In intercropping, one crop is sown with or after another crop and harvested before it. In interplanting the second crop is sown shortly after another and also harvested after it. As the duration of "available" water becomes more limiting, the range of crops that can be grown becomes less. As the growing season extends, relay crops become possible.

India

Within SAT India 57.2% of the land is under cultivation - for India as a whole the figure is 44.6%, the highest proportion of the total land area for any country in the world (IARI, 1970). In the low rainfall (400 to 1000 mm), unirrigated areas grain legumes are sown on a much reduced scale of cereals (Table 6). This area represents nearly 36% of the net sown area in the country, covering 47 million ha. There are wide inter-regional variations of cropping patterns however. For example in some districts in Gujarat and Andhra Pradesh the percentage of land under groundnut can be nearly 30% of the total cropped area.

Some of the crop mixtures on three broad soils types in SAT India are discussed.

Black Soils

Black cotton soils, also called vertisols, are formed from a variety of rocks which include traps, granite and gneisses, particularly those rich in lime feldspars. Thus they are usually rich in bases including potassium, calcium and magnesium. In the black soils, the montmorilinitic and beidelite types of clay predominate and impart the characteristic swelling and shrinking properties and a high water-holding capacity. The pH ranges from 7.0 to 8.5 and the lime content from 1 to 10%. Owing to their formation under semi-arid tropical conditions, the soils are low in organic matter (0.4 to 0.8%) and are usually deficient in nitrogen, phosphorus and sometimes zinc.

Black soils are found mainly in the central region of India and total about 60 million ha with 54 million ha rain-fed (non-irrigated) of which about 30 million ha would be under cultivation (Singh, 1974).

The cropping patterns followed in the various black-soil regions may be grouped as follows:

(a) Cropping in monsoon season only. This pattern is followed on shallow and medium textured, moderately deep, black soils by utilizing the available water in the rainy (kharif) season. Less than 40% of the black soil appears to fall in this group (Singh, 1974). The most common cropping mixtures and sequences are as follows:

Sorghum + pigeonpea + sesamum (intercrop)
Pearl millet + pigeonpea + sesamum (intercrop)
Sorghum + pigeonpea - fallow (2 year rotation)
Sorghum + pigeonpea - groundnut (2 year rotation)
Sorghum + pigeonpea - cotton (2 year rotation)
Cotton - sorghum + pigeonpea - groundnut (3 year rotation)

The pigeon-peas which are grown in mixtures with sorghum are late maturing and are harvested by February.

(b) Cropping in post-monsoon season only. It is estimated that over 50% of the rainfed, deep or moderately deep black soil or more than 15 million ha is fallowed during the monsoon and then cropped during the post-monsoon season. The area under monsoon fallow is decreasing (Singh, 1974).

Common cropping sequences are:

Fallow - sorghum + oilseed mixture (intercropping)
Fallow - chickpea (continuous)
Fallow - wheat (continuous)
Fallow - wheat - fallow - chickpea (2 year rotation)
Fallow - wheat - fallow - linseed (2 year rotation)

Initial observations at ICRISAT indicate that if suitable soil, water and crop management systems are used, a substantial increase in production could be realized by cropping some of these fallowed lands during the monsoon season. With short-season monsoon crops there is a possibility of relay or double cropping in the medium to high rainfall area (>750 mm).

(c) Cropping during both monsoon and post-monsoon seasons. In some deep black soils, a monsoon crop is grown during the rainy season followed by catch crops of pulses or oilseeds grown on residual moisture.

If rains are sufficient, wheat or a post-monsoon sorghum-oilseed mixed crop is grown. Such double cropping appears to be practised on less than 10% of the black soil area.

Common sequences followed are:

Monsoon crop	Post-monsoon crop
Sorghum	- pulse (chickpea or horse gram)
Sorghum	- safflower
Groundnut	- safflower
Pulses (Cowpea, Green gram, Black gram)	- wheat
Pearl millet	- wheat
Cotton	- sorghum + safflower

Some of the fast establishing, short season pulses - *Vigna radiata, V. mungo, V. unguiculata* complete their growth in 60 - 80 days and are sometimes grown in the kharif (monsoon) season before the rabi crop is taken. For example cowpeas at Sholapur in 1973-74 sown at the beginning of the kharif yielded 1.72 t/ha and *Vigna mungo* in 1974-75 yielded 1.57 t/ha. The normal rabi crops were then taken (CH. Krishnamoorthy, pers. comm.). Current experiments at ICRISAT are examining mixtures of these pulses with pigeon pea and maize sown at the beginning of the kharif. The short season pulses will be harvested first followed by maize, leaving pigeonpea to complete its growth by 160-180 days. Such crop mixtures use land otherwise bare early in the season between the longer duration crops. Whether the yield of maize and pigeonpea suffer remains to be seen. Experiments at ICRISAT with pigeonpea - *Setaria italica* mixtures have given promising results (see section 5.4).

Red Soils

The red soils are usually developed from ancient granites or gneisses, are usually low in nitrogen, phosphorus and sometimes zinc with medium to high potassium level and a pH ranging from 5.5 - 7.0. The clay fraction is rich in kaolin. Red soils range from shallow to medium in depth and have a relatively low water-holding capacity. Crops are grown only during the monsoon season. Sorghum, groundnut, setaria and ragi or finger millet *(Eleusine coracana)* may be grown as pure crops or as mixtures with each other or with pigeonpea, pearl millet, castor, cowpea and cotton.

Important cropping mixtures and sequences followed in red soils are:

Cotton	+ setaria (intercropping)
Sorghum	+ pearl millet + pigeonpea (intercropping)
Finger millet	- groundnut or horsegram (two year rotation)
Sorghum	- pulses (two year rotation)
Finger millet	- pulses (two year rotation)
Sorghum	- groundnut - cotton - setaria (4 year rotation)
Finger millet	- continuous

In seasons with late rains in September, crops like horsegram, sesamum and safflower are grown following early-maturing monsoon crops.

Table 7. Cropping Systems in Two Locations in Semi-Arid Rainfed Northern Nigeria[a]

	Variable	Saria area	Sokota area
Location:	Latitude (N)	11° 11'	13° 01'
	Longitude (E)	7° 38'	5° 15'
Climate:	Length of growing season (days)	174	143
	Total rainfall (mm)	1040	760
Population density (persons/sq km)[b]		1024	916
Farm land[c]:	Total hectares surveyed	360.5	350.7
	Percent in: Fallow	19.0	3.4
	Sole crops	13.4	6.3
	Crop mixtures	67.6	90.3
Percentage of crop mixture area in:			
	Two crop mixtures	50.5	23.4
	Three crop mixtures	28.4	51.0
	Four crop mixtures	14.5	23.9
	Five or More crop mixtures	6.6	1.7
Number of crops grown per family[c]		8.0	5.7
Percentage of adjusted hectares in:[d]			
	Millet	20.4	33.2
	Sorghum	31.6	19.2
	Groundnuts	11.8	(2.4)
	Cowpeas	12.1	30.7
	Cotton	10.5	-
	Total devoted to major crops	86.4	83.1

a. Taken from Norman (1976)
b. Includes land not suitable for cultivation, residences, etc.
c. Includes both rainfed and lowland fields.
d. In calculating the total adjusted hectares of crops each crop constituent was allocated an area calculated by dividing the number of hectares of the crop mixture by the number of crops in that mixture.

Alluvial Soil

These soils usually possess good physical condition, are easily tilled and are moderately permeable. The water-holding capacity is relatively low compared with that of the black clay soil. These soils are usually low in organic matter and deficient in nitrogen and sometimes low in phosphorus and zinc. Potash may also become deficient after prolonged intensive cropping.

The major crops grown during the monsoon season are pearl millet, sorghum, pigeonpea and groundnut, and during the post-monsoon season are wheat, barley, chickpea, mustard and rape. The cereal crops are usually grown as a mixture with an oilseed or legume crop.

The common cropping patterns followed are:

Monsoon fallow - wheat (one year sequence)
Monsoon fallow - barley (one year sequence)
Pearl millet - chickpea (one year sequence)
Pearl millet - barley (one year sequence)
Monsoon fallow - chickpea -monsoon fallow - sorghum + sesamum (2 years)
Sorghum + grazing - monsoon fallow - wheat (2 years)

Guar *(Cyamopsis tetragonoloba)* has been successful as a forage cum grain crop as a replacement of the fallow - wheat sequence and has added nitrogen to the soil reducing the requirement of the wheat crop for N fertilizer.

Africa

In both shifting cultivation systems and more intensive production sequences with shorter fallows in the semi-arid tropics, legumes play a minor but important role. Legumes are infrequent in the traditional bush regrowth, with grasses predominating. Some legume shrubs develop quickly but their nodulation status is not known (Jones and Wild, 1975). The crop mixtures vary in different parts of Africa. In East Africa pigeonpea and maize mixtures are found, as well as *Phaseolus vulgaris* and maize in both the long and short rainy season. Pigeonpeas are usually long duration maturing during the second rainy season. Peas and *P. vulgaris* beans may be planted in kitchen gardens among banana groves (e.g. Ruthenberg, 1975).

About 75% of the 42 million population of semi-arid Nigeria derive their living from agriculture. In general indigenous cropping systems prevail. Surveys show that only a small percentage of the farm land is in solid crops (c 13% in Zaria and 6% further north in the Sokoto area). Crop mixtures occupied 68 and 90% of the farm land respectively in these areas, with 2 - 3 crops usually involved but with up to 5 or more sometimes found (Table 7). An example of the area under various mixtures is given in Table 8 (Norman, 1976).

A complex interaction of physical and biological factors, as well as socio-economic factors determine this cropping pattern. Farmers attempt to grow other crops than millet, the most reliable crop as rainfall and length of season decrease, as a bonus. Spacings, and apparently yield, of the millet plants are not affected by the intercrops. As the growing season shortens, cowpeas become more popular than the longer season groundnuts. Even if the crops fail to produce a grain yield, the dry vegetative plant has a value - cowpea as animal fodder, sorghum stalks for fuel and fencing. As the season becomes longer the complementary nature of the intercrops becomes more important. The pattern tends to be to plant millet and sorghum mixtures at the beginning of the season, followed by cowpea planted among the sorghum

Table 8. Major Crop Enterprises in two locations in semi-arid Northern Nigeria*

Crop enterprise	Percentage of land	
	Zaria area	Sokoto area
Sorghum	8.4	0.5
Millet/Sorghum	25.8	0.9
Millet/cowpea	-	18.6
Millet/sorghum/groundnuts	5.0	-
Millet/sorghum/cowpeas	3.9	40.6
Millet/sorghum/groundnuts/cowpeas	5.4	1.2
Millet/sorghum/cowpeas/red sorrel	-	13.8

*All crop enterprises were grown in systematic planting patterns. Taken from Norman, 1976.

just before or after the millet is harvested, or after weeding the cereals. No special land cultivation is required and the spreading growth of the cowpeas makes further weeding unnecessary. Cowpea yields can be severely reduced if the season ends early, and tend to be less than for sole crops, but little research has been done to select more suitable cowpea varieties for this situation (Steele, 1972). In farmers fields, sorghum and groundnut yields also tend to be lower than in sole cropping, probably because sorghum stands are fewer, and because shading by the taller crops in the mixture has an adverse effect on groundnut. The longer duration groundnuts tend to be planted as a sole crop.

Such farm mixtures seem to make maximum use (coverage) of the land and the labour involved in preparing and ridging it, as well as spreading the labour input more evenly over the season, and helping to alleviate peak demands for labour. An evaluation of the returns per unit of labour input and monetary returns per hectare clearly indicates the superiority of mixtures over sole crops - 20% higher return for mixtures in the Zaria area, and 146% higher in the Sokoto area (Norman, 1976).

In SAT Africa west of Nigeria (e.g. Senegal) the "shifting cultivation" or bush fallow system has given way to a rotation involving shorter fallows - with the proportion of land in fallow being as low as one quarter. The cycle of cropping thus takes place more frequently. The fallow has changed from secondary forest or wooded savannah to grass fallows with some bush sprouting from the stumps. The soil fertility under this system is gradually declining, although the introduction of cash crops such as groundnut and cotton has increased the income level, albeit to a still low level. The cropping systems

involve mainly sorghum and groundnut generally in sole crops (c. Charreau, pers. comm.)

In traditional systems in Zambia and Northern Rhodesia, finger millet *(Eleusine coracana)* is the first crop sown by broadcasting in poor sandy soils after fallow. A little sorghum and sesame may be mixed and later *Phaseolus vulgaris*, sweet potatoes and cassava planted around the edge of the millet. Cropping may continue for two to four years with groundnut and beans planted in the second year followed by sorghum and cassava (Trapnell, 1943).

In parts of Malawi and southern Tanzania farmers make use of the organic matter in the grass fallow by not burning it but by hoeing it into buried mounds at the end of the rainy season. A crop of beans *(Phaseolus vulgaris)* or cowpeas is raised in the mounds. In the next season they are planted with finger-millet or maize and beans. Weeds are hoed out into new mounds in which groundnuts, beans or cowpeas are sown. In subsequent seasons maize or other cereals are sown in the mounds along with a quick maturing, climbing bean variety. After some years the land is planted to cassava or pigeonpea before being left to revert to bush fallow. These farmers apparently appreciate the benefits of organic matter for legume growth (eg. Dart, Day, Islam and Dobereiner, 1975).

Northeast Brazil

Maize, dry beans (mainly cowpeas and some *Phaseolus vulgaris)*, cassava and bananas are the major food crops. Although the area under cultivation increased from 4.5 to 10.9 million ha between 1950 and 1969, there was no appreciable increase in average crop yield during this period. Most of the maize and beans, with or without cassava are sown as a mixed crop (broadcast) or intercropped (alternate rows). Small amounts of castor beans, pumpkins and palma (a cactus for forage) may also be included. Again a bush fallow breaks the cropping sequence and restores fertility.

Mixed cropping

Crop mixtures judiciously chosen so that the periods of maximum growth of each crop do not overlap theoretically make better use of available sunlight, water and nutrients. Farmers know this and intercropping is common throughout the semi-arid tropics, but little research has been done to maximise production of these systems. Often the grain legume component is a bonus which does not reduce the companion - usually cereal - crop yields. Spreading legumes such as cowpea may help smother weeds; for other species such as pigeonpea in India, the widely spaced intervals between rows, with 4 or more rows of sorghum or cotton intervening may reduce pod borer attack of the pigeonpea. If borers are controlled high yields of pigeonpeas and companion cereal crop can be obtained with closer row spacing.

Table 9 shows the comparative effect of different intercrops on pigeonpea yields when pigeonpea was planted at 75 cm row spacing, with the intercrop between these rows. *Setaria italica* was the least

Table 9. Effect of Different Intercrops on Pigeonpea Yields, ICRASAT 1975-76

Intercrop	Pigeonpea grain yield (t/ha)	
	Black Soil	Red Soil
Pigeonpea	1.73	1.28
Cowpea	1.55	1.01
Soybean	2.13	1.66
Sorghum	2.27	1.25
Pearl millet	1.97	0.96
Setaria	2.55	1.76
No intercrop.	2.53	1.98
LSD (05)	0.63	0.40

competitive intercrop, and still produced over 3.5t grain/ha which was not significantly different from the sole crop yield. Thus the aggregate yield for the intercrops was about double that of the same species as sole crops sharing the same total area as the intercrops. For the spreading, medium duration pigeonpea which is slow to establish, the rapidly establishing, early maturing, upright, nonratooning setaria was an excellent intercrop. Other experiments have demonstrated the increased monetary return from several mixed cropping combinations (Krantz *et al.*,1976). The gross value of the grain plus stubble, used as fodder, is an important consideration in determining profitability of crop mixtures.

Current research at ICRISAT is examining the effect of different architectural arrangements on yield of intercrops based on pigeonpea by manipulating planting arrangements, plant type and numbers of plant species involved.

Intercropping is the "natural" way to sow crops for many farmers. It seems likely that they would more readily accept improved practices related to intercropping rather than sole cropping.

ROLE OF LEGUMES

In soil fertility

Since very little N fertilizer is used in the semi-arid tropics, soil fertility is largely maintained by biological nitrogen fixation, and mainly by legumes. Fields close to settlements may receive organic residues or farm yard manure. There are however, virtually no field estimates of the amounts of nitrogen involved, except insofar as a bush fallow restores the soil fertility for subsequent crops (Jones and Wild, 1975).

Annual grain legumes could enrich the soil nitrogen if plant parts other than the grain were returned to the soil. With the vegetative material being removed for feeding to animals, or being removed when the whole plant is harvested for thrashing, usually near the home, this is often not the case. It is sometimes assumed that the legume is not a net depleter of soil nitrogen, the amount of fixed nitrogen in the roots etc. remaining in the field, balancing the mineral nitrogen uptake and removal in the harvested parts of the plant.

A field experiment at Katherine in SAT Australia, examined the nitrogen fixation by Townsville stylo *(Stylosanthes humilis)*, cowpea, groundnut, and guar *(Cyamopsis tetragonoloba)* over three successive wet seasons. Stylo and cowpea were cut for hay and removed, for peanut and guar the haulm was returned to the soil after the nuts and grain were harvested. Townsville Stylo and guar fixed 220 Kg N/ha over the 3 years of cropping, peanuts 123 Kg/ha and cowpea 269 Kg/ha. Stylo fixed annually 93 Kg/ha in the second and third seasons. Because of the crop removal only stylo and guar maintained the original soil nitrogen status, with guar returning more N to the soil than was removed. The results indicated that stylo grazed with a return of 85% of the ingested nitrogen to the soil, would substantially increase soil nitrogen. Cowpea and groundnut did not enhance the soil N status (Wetselaar, 1967). Martin (1970) also found a decline in soil nitrogen status after three seasons of groundnuts in the Niari valley, Congo (Brazzaville).

However, experiments at the Institute of Agricultural Research, Samaru, Northern Nigeria, indicate that groundnuts can substantially benefit yields even if the cereal received nitrogen fertilizer. Maize, even when fertilized with 168 Kg N/ha, yielded more following a single groundnut crop than after N fertilized cotton or sorghum. All above ground parts of the preceding crop were removed at harvest. Maize yields with no nitrogen fertilizer additions were 44% greater after groundnuts (3,440 Kg/ha) than after the sorghum (2,390 Kg/ha) (Jones, 1974). Grain legume yields should be thought of in terms of the whole plant, and perhaps in some agricultural systems, plants with lower grain yields but commensurately greater potential for enriching soil fertility, might be the plant type to seek.

Pigeonpea has great potential in this regard, especially as its deep root system also cycles nutrients other than nitrogen from deeper soil regions to the surface. For a crop like pigeonpea where leaf fall through the season is considerable, quite a large part of the nitrogen uptake of the plant is returned to the soil (Table 10). If the assumption is made that the nitrogen in the root system plus stems is equivalent to the mineral N uptake by the plant, then the N in fallen leaves and grain would be equivalent to the nitrogen fixed, and amount to c 100 Kg N/ha season (A. Narayanan & R. Sheldrake, ICRISAT unpublished observations, 1976).

It is important in soil nitrogen balance studies with legumes that the nodulation status of the crop is observed. Poorly nodulated legumes will obviously fix little nitrogen.

Intercropping experiments with *Desmodium* spp forage legumes would

Table 10. Dry Matter Production and Nitrogen Uptake by Pigeonpea (CV ST-1) at Harvest. No N Fertilizer supplied*.

Plant Part	Dry matter Kg/ha	Nitrogen %	Nitrogen Kg/ha
Seed	1455	3.54	54.4
Pod wall	850	1.12	9.5
Stem	4318	0.44	19.0
Fallen leaves	2233	1.63	36.4
Total	8856	-	119.3

*(Narayanan & Sheldrake, ICRISAT, unpublished results 1976).

suggest that little nitrogen is excreted by legume roots and nodules for uptake by the associated, usually cereal crop (eg. Whitney & Kanehiro 1967; Whitney, Kanehiro & Sherman, 1967; Whitney & Green, 1969). No experiments have been reported for SAT legumes. Benefits to the growth of the non-legume crop may partly occur through the sparing effect of the legume on the availability of nitrogen for the associated crop. Nodulated legumes usually take up N less rapidly from soil than non-nodulated plants (Henzell and Vallis, 1975). Thus in intercropping versus associated sole cropping, for the same total planted area, the cereal intercrop would have access to a larger pool of available N than whensole cropped, and the rapid depletion of available soil N by the intercropped cereal would tend to enhance the rate of nitrogen fixation by the legume since mineral N generally decreases N_2 fixation.

In the Savanna fallows in SAT Africa, leguminous trees and shrubs occurs, although they have low nitrogen contents, suggesting that little nitrogen fixation is occuring (Nye, 1958). However some nodulated trees apparently fix much nitrogen that can benefit subsequent or associated crops. Stands of the nodulated non-legume *Casuarina* fixed 60 Kg N/ha in Senegal (Dommergues, 1966), and in India plantings of *Casuarina* are used for wood production and for increasing soil fertility. *Acacia albida* is a traditionally protected tree in much of the drier savanna in central and West Africa. It is leafless during the rainy season and hence does not shade crops grown underneath it, and these crops give markedly higher yields than plants outside the trees' zone of influence. Soils under these trees are richer by 25-100% in organic matter and nitrogen, as well as exchangeable calcium and total exchangeable bases (Charreau and Vidal, 1965; Radwinski and Wickens, 1967). Trials

Table 11. Annual Return of Elements to Soil in *Acacia albida* Litter*

Estimates per tree or per ha (in Kilograms)		Dry Organic Matter	N	P_2O_5	K_2O	CaO	MgO
Per tree	- Mean surface under canopy: 231m^2	267.5	4.3	0.2	2.1	7.2	1.6
Per ha	- Maximum stand density (1)	11,583	187	9	91	311	69
	- Mean stand density (2)	5,350	69	4	39	112	31

(1) Dense population (44 trees/ha); continuous cover; no removals
(2) Medium population (20 trees/ha); partial removals (50 percent for nitrogen, 10 percent for the other elements)
*Derived from Jung, 1967 by C. Charreau

with cereals suggest an additional 15-20 Kg N/ha/season is mineralized in the ambience of these trees (Dancette & Poulain, 1968), and yield increases of 152% can be obtained with pearl millet; some of this yield increase is due to enhanced mineral nutrition as fertilizer addition reduces the effect of the tree. The annual return of nutrients to the soil in *A. albida* litter is shown in Table 11. The pods are also valuable cattle fodder with 7% digestible protein, and a dense stand can produce 2,500 Kg pods/ha/season with much more feeding value than any other local fodder including groundnut haulms (Charreau and Nicau, 1971).

In SAT India, the small tree *Prosopis juliflora* also enhances growth of crops growing near it and the pods are relished by cattle.

Potential exists in SAT Africa, for substituting a forage legume based pasture for the currently used bush fallows. *Stylosanthes* spp will be important here. If a better integration between animal and plant food production could be achieved, new agricultural systems would be easier to adopt (Jones and Wild, 1975). In India scope exists for fodder production based on legume-grass mixtures on non-arable land, and 'common' land currently producing little with virtually no management inputs. Restricting animal access to such areas has an enormous impact on productivity. This increased yield potential should make it economic to produce improved fodder/forage crops by establishing improved legume and grass species (see Jones, These Proceedings). Early observations suggest species such as Siratro *(Macroptilium atropurpureum)* and *Stylosanthes hamata* are well adapted to SAT India.

One of the difficulties in establishing new cropping systems is that they often lack some "minor" advantages of the indigenous system, and this makes it difficult to convince farmers of the superiority of

the new approach. After all, indigenous systems have evolved over long periods of time and after much empirical experimentation by the farmer. They are well adapted to the climate and currently available inputs and are stable in yield; only population pressure makes it necessary to develop new systems.

Some of the miscellaneous uses of leguminous trees and shrubs, many of which occur naturally, are important factors in attempts to substitute new systems of management in place of the bush fallow. In SAT Africa they provide structural material and mortars and pestles (*e.g. Parkia* spp and *Tamarindus* spp), fuel (e.g. *Acacia* spp), shade and ornaments (*Abrus precatorius Tamarindus indica, Canavalia* spp, *Dolichos* spp), hedges and fence posts (e.g. pigeonpeas), mucilages, gum arabic and tanning material (e.g. *Acacia senegal*) medicines (e.g. *Lupinus arkorous, L. Persilus, L. barbiger, Piliostigma thonninglii , Tephrosia purpurea*) and religious artifacts (e.g. *Voandzeia subterranea*). Such legumes are often protected in the wild, and even cultivated. They would need to be propagated elsewhere or acceptable substitutes provided if other plants are to be grown in their place. This would undoubtedly involve more work for the farmer and for any new system to be adopted it would need to be substantially more attractive than the present one of minimal management of such useful plants.

Legumes can enhance soil fertility in the semi-arid tropics; ways need to be found to increase this potential and their use for this purpose.

Role of legumes in human nutrition

Taking a calculated average daily requirement of calories, protein and lysine, of 2373 K calories, 42.7 g and 1.36 g, the amounts available in an average Indian diet are mostly deficient in calories. Where protein deficiency occurs it is much less severe than the calorie deficiency. Data for Northern Nigeria, Kenya, Northeast Brazil, and North East Thailand indicate that the diets seemed adequate for these nutrients. In Tanzania and Jamestown in Ghana, calories were again deficient with adequate protein levels (Ryan, Sheldrake & Yadav 1974, 1975). Since very little meat is eaten in the semi-arid tropics, pulses constitute an essential part of the diet providing protein and complementing the amino acid availability from other sources to provide the desired levels of essential amino acids. For example in northern Nigeria, with diets based on sorghum, millet and cowpeas, the cowpeas provide protein, lysine and nicotinic acid giving a diet in which the ratio of protein to total calories is comparable to the wheat based diets of European countries (Autret *et al.*, 1968). In countries where groundnut is consumed as a food, it supplies at least 10% of the calculated minimum protein requirements per day. Although groundnut is moderately deficient in methionine some breeding lines in Florida have adequate methionine contents, and up to 100% greater than the average level.

Since average calculated availabilities of nutrients do not necessarily reflect the actual distribution, some groups in the community will be vulnerable, especially children and lactating mothers where the nutritional requirements differ from the working adult. However even

in low income Indian families, surveys indicate that pulse intake balances the nutritional requirements although calory intake is deficient. With increasing income, people in the semi-arid tropics eat more pulses as a preferred item in the diet. Demand for pulses will increase with increased productivity of cereals because the price reduction of cereal grains likely to be associated with their increased availability will leave more income available for purchase of pulses. Thus intake of both cereals and pulses is likely to increase.

Legumes thus have a continuing and essential role in the nutrition of people in the semi-arid tropics.

Acknowledgment

We are greatful to our colleagues at ICRISAT in the Pulse and Groundnut Improvement Programs and Economics Department who have generously allowed us to draw freely from their writings and unpublished results.

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THE ROLE OF LEGUMES IN SOUTH AND SOUTHEAST ASIA

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INTRODUCTION

People in South and Southeast Asia rely heavily on cereals and legumes for their major nutrients. FAO statistics (FAO, 1975) clearly show that protein needs are inadequately met and that vulnerable groups are suffering from both calorie and protein deficiency. At least, 90 million people, mostly children, are suffering from moderate to severe malnutrition. Greater legume production is necessary to provide even the generally inadequate protein and vitamins to the 2,200 million people living in the region. Thus, the vital role of legumes in this region is evident both as a source of protein in Asian diets and from the need to diversify agricultural production.

Legumes also function in many ways in soil conservation and as nutrient supply factors in soil management. Legumes (*Canavalia, Pueraria)* are used as the major cover crops in rubber and oilpalm production. Leguminous trees such as *Leucaena* enrich the soil with organic matter in tropical forests. (Frederick, 1976). Legume crops are used in multiple cropping schemes as a green manure component to supply organic matter and nitrogen to the soil and as a cash crop for higher farm incomes (Cheng, 1975). Some legumes provide fibers *(Crotalaria)*, and the natural Indigo dye comes from a legume *(Indigofera)*. Pasture legumes are an important component in animal protein production in those areas where either population densities, rainfall, or both are low enough to permit the use of land for extensive meat and dairy production.

Lists of the major species of tropical legumes in current cultivation with their taxonomy, principal uses, and general ecology have been published. (Rachie and Roberts, 1974; Whyte, *et al.*,1953). Legumes are usually presented in four broad categories: food legumes, oil seeds, forage and cover crops, and miscellaneous. Several recent reviews have analyzed the improvement of both general and food species of legumes (Rachie and Roberts, 1974; TARC, 1972).

The purpose of this paper is to discuss the role of legumes in South and Southeast Asia and their contribution to agriculture and the the quality of life for the people in both rural and urban communities.

Emphasis will be placed on productivity and on nutrition. Since the role of legumes at present implies that change is underway, we will also discuss potentials for improvement, some of the major current problems limiting the role of legumes, and the breeding and management techniques which may bring about the desired improvements.

Table 1. Area, yield and production of pulses in Asia, North America and the world. 1961-65 and 1974. (FAO Production Yearbook, 1974)

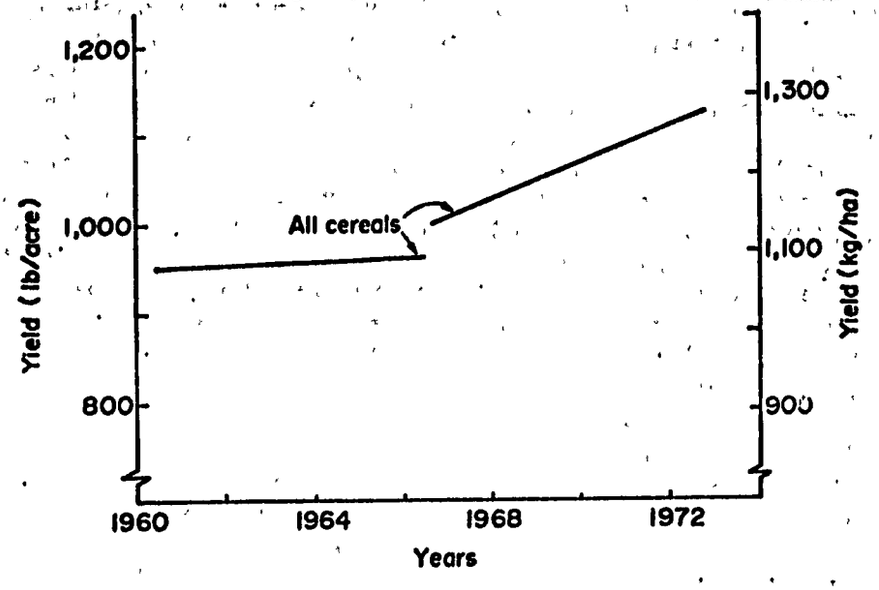
Year	1961-65	1974
Asia		
Area harvested (10^6ha)	37.4	36.4
Yield (kg/ha)	595	595
Production (10^6 t)	22.2	21.6
North America		
Area harvested (10^6 ha)	3.3	3.3
Yield (kg/ha)	730	911
Production (10^6 t)	2.4	3.0
World		
Area harvested (10^6 ha)	67.5	66.3
Yield (kg/ha	621	666
Production (10^6 t)	41.9	44.1

Table 2. Present situation of mungbean in Asian countries.[a]

Country	Ara	Ranking among Legumes	Productivity	Price	
				Mungbean	Rice
	(ha)		(kg/ha)	(US$/kg)	
Taiwan	4,705	3	672	0.41	0.45
Philippines	38,450	1	419	0.50-0.80	0.25-0.30
Indonesia	29,000	3	600	0.72	0.38
Thailand	243,000	1	780	0.20	0.20
Burma	184,000	4	511	0.40-0.50	0.30
Bangladesh	18,840	5	750	0.50-0.75	0.33-0.50
India	1,300,000	3	380	0.25-0.30	0.17-0.18
Sri Lanka	10,500	1	617	0.82	0.32
Pakistan	68,440	3	550	0.30	0.15
Papua New Guinea	All imported			1.50	0.50

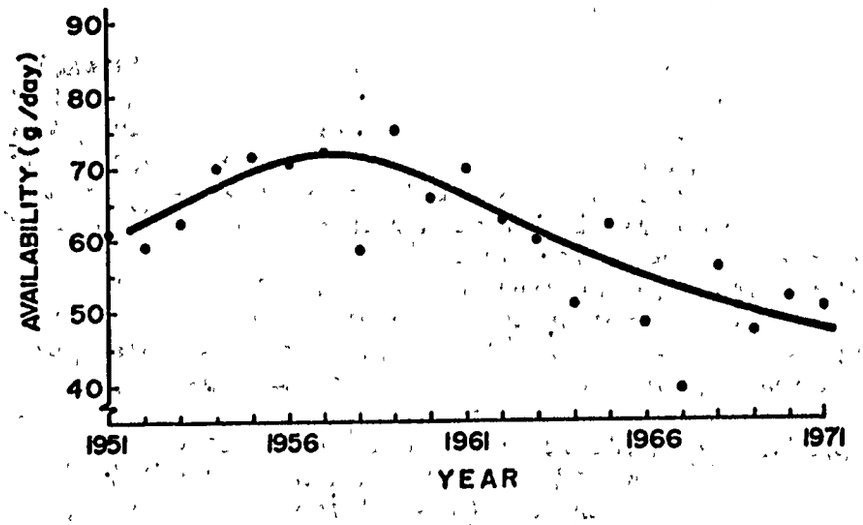
[a] Data extracted from national reports submitted to FAO at the IAEA's Regional Legume Seminar, Dec.8-12,1975, Colombo, Sri Lanka.

FIGURE 1. Food grain yields – developing countries.



Linear regression of food grain yields for developing countries, time periods 1960 - 1966 and 1967 - 1972. *(After Chancellor and Goss, 1976).*

FIGURE 2. Pulse availability, India, 1951–1971.



Availability, g/caput/day. *(After Singh, 1974).*

Role of Legumes in Agricultural Production

Recent studies (IFPRI, 1976) of food production prospects for the world predict that cereal grain production will be about 100 million tons short by 1985. Others predict that the average per capita diet will improve slightly by 1985 and then remain stable (Chancellor and Goss, 1976) although requiring an increase of about 88 % in calorie production over that of 1970. The difference between these forecasts depends on the rates of change used to estimate the production increase. Historically, food production increased about 2.1% annually, whereas a changed rate of grain yield increase estimated at 3.3% or so has been observed. Chancellor and Goss (1976) have shown that yield trends for major cereal crops have changed in recent years as a result of the introduction of "Green Revolution" technology (Fig. 1).

The per caput availability of legumes, however, has declined from 60 g/day to 50 g/day over the 20 year period, 1950-1971 in India (Fig. 2). Farm production of legumes is being replaced by cereals since there are now increased yields and better returns from growing cereals using new varieties and management. The uncontrolled increase in population is the other major factor resulting in reduced availability.

Research on pulses has been neglected and as a result the growth rate for pulse yield from 1950 to 1971 in India was only 0.03% (Berg, 1973). In recent years owing to the input by the International Soybean Resource Base (INTSOY) in Illinois, acreage, production, and nutritional use of soybean in India may again be on the rise.

Total world area planted to pulses has decreased, whereas production has remained unchanged since 1960 (Table 1). The shortage of legumes in India has been reflected in price, which has risen sharply since 1960 (Singh, 1974). This trend is particularly damaging for India where many vegetarians use legumes as a principal source of protein. It is also reflected in other Asian countries as shown by the higher price of mungbean relative to the price of rice (Table 2).

The role of legumes in agricultural production is small relative to that of cereals, but the role of legumes in human nutrition is more important than their relative acreage or production volume (harvested crop) indicate. Representative percent ages of total crop acreage and production are presented for selected Asian countries in Table 3. Legumes normally constitute only 1 to 3% of planted crop area and even less of production.

Of the food legumes considered here, only 4 to 5% of production enters world trade, whereas 20 to 40% of groundnut production enters world trade each year (Ryan, 1974; FAO, 1974). Soybeans, of course, are extensively grown and traded, and provide a major part of the export earnings of the United States and Brazil. In 1975, the United States produced 41.4 million tons of soybeans out of the 68.4 million tons produced in the world. In South and Southeast Asia, Indonesia is the largest producer with only 0.56 million tons. Such low production can be attributed to the low acreage devoted to soybean

Table 3. Comparison of legumes, cereals, and root and tuber crops in selected Asian countries as percentages of total land area and production for food crops.[a]

Country	Legumes		Cereals		Root & Tubers	
	Area	Production	Area	Production	Area	Production
	%					
Philippines	1	0.3	95	86	4	14
Thailand	3	1	92	71	5	28
Indonesia	9	2	77	61	14	37
Bangladesh	3	1	96	92	1	7
India	24	8	74	82	2	10
Sri Lanka	1	0.3	87	75	12	24

[a]Source: FAO, Production Yearbook, 1973, Rome.

Table 4. Soybean production cost and returns for three Asian countries.

	Thailand[a]	Philippines[b]	Taiwan[c]
Field size (ha)	0.96	1.20	0.70
Yield (kg/ha)	872.00	814.00	1,700.00
Production costs ($/ha)			
Cash	41.50	75.00	366.00
Non-cash	30.50	115.30	163.00
Total	72.00	190.30	529.00
Price (US$/kg	0.10	0.33	0.29
Net return/ha (US$)	17.30	80.00	-27.50
Net return/kg (US$)	0.02	0.10	- 0.02

a. Chiang Mai b. Southern Cotabato c. Pingtung-Kaohsiung

Source: AVRDC, 1976.

as well as deplorably low yields due to a lack of improved varieties and suitable technology to boost production (Schanmugasundaram, 1976).

Legumes have a minor role in tropical cropping systems even where regularly included in multiple cropping patterns. In most of the countries, legumes are planted on marginal to poor lands or in seasons with little rainfall and are grown with minimum management.

Taiwan is an exception to the above as cropping is intensive, and legumes such as pea, snap bean, mungbean, adzuki bean, groundnut, soybean, etc. form part of intensive multiple cropping systems. In Southern Taiwan, two rice crops and one legume (either soybean, adzuki bean, or mungbean) are normally grown and harvested each year (Chandler, 1974; Cheng, 1975; McKenzie, 1974; Shanmugasundaram, 1974, 1976a, 1976b). In 1975 the average soybean yield in this "No-tillage rice-stubble cultivation" equalled the average yield for the world, 1.5 tons/ha (Shanmugasundaram, 1976c). However, the soybean area and production is declining due to the pressure of large volumes of low-cost, imported soybeans (AVRDC, 1976). Mungbean production also has declined (Cheng, 1972) in spite of the high unit price (US$0.84/kg) owing to low yields and low net returns to the farmer.

Soybean production in Thailand has increased sharply in recent years (1968 to 1972). Philippine soybean production has remained low and stable (k,000 t/yr), while Taiwan's production declined by 20% during the same period. Analysis of production costs and returns show strong positive returns for farmers in the Philippines and Thailand, but losses per hectare and per farm in Taiwan (Table 4). Thailand and the Philippines have relatively large farms, abundant inexpensive labor, and fair to good yields indicating good potential for expansion. In both countries, many costs are met on a non-cash basis, but marketing problems limit expansion of soybeans as a cash crop. Taiwan's small farm size and limited costly labor require significant price adjustment or yield increases to reverse the downward production trend (Menegay and Huang, 1976).

Much progress has been made in tropical pasture agronomy in the past 20 years primarily in Australia, New Zealand, and Hawaii. Legumes will continue to be used in pasture mixtures as long as extensive land areas are available for use. When human population densities exceed about 100 persons per sq. km, pasture production invariably gives way to tilled systems of agriculture. Asian countries offer great opportunities for increased pasture and food legume utilization.

Pasture legumes such as Greenleaf desmodium *(Desmodium intortum)*, Townsville stylo *(Stylosanthes humilis)* and Siratro *(Macroptilium atropurpureum)*, which are being used in the Pacific Islands, can be tried in the potential pasture areas in Asia.

Earlier studies of utilization of *Leucaena* (Kinch and Ripperton, 1962) showed the high potential of this species as a high protein feed, but practical utilization has lagged owing to management problems. Revival of interest in other species of *Leucaena* have resulted from identification of more productive cultivars (Brewbaker, 1975).

Role of Legumes in Human Nutrition

Brown (1974) estimated that one billion people suffer from protein and vitamin malnutrition, at least during some part of the year. In recent studies, one-quarter to one-third of 200,000 children examined clinically in developing countries were judged to be malnourished due to lack of either protein, vitamins, or both. This was "point-prevalence" and in some areas malnutrition approaches 100% in the first five years of life (Dinning, 1975). If these deficits are to be corrected, more attention must be given to the role of legumes.

Among the 13,000 species of legumes, about 20 are eaten in significant quantities by man directly as food. Soybean is easily the most important of these, especially in China, Japan, Korea, Taiwan, and Indonesia. Mungbean *(Vigna radiata)*, black gram *(V. mungo)*, chickpea *(Cicer arietinum)*, pigeon pea *(Cajanus cajan)*, lentil *(Lens esculenta)*, dry beans *(Phaseolus vulgaris)*, lathyrus pea *(Lathyrus sativus)*, broad bean *(Vicia faba)*, cowpea *(Vigna sinensis)*, groundnut *(Arachis hypogaea)*, and dry pea *(Pisum sativum)* are some of the important pulses in India, Thailand, Pakistan, Bangladesh, Philippines, Sri Lanka, and Iran. Among these, groundnut has the highest calorie amount per unit weight largely owing to its high fat content. Aykroyd and Doughty (1964) and others have produced detailed studies of the production, consumption, and quality factors in legumes which affect human nutrition. The nutritional values for selected legumes are presented in Tables 5 and 6.

The diets of many Asians and westerners (Americans) differ substantially in their composition (Oyer, 1976). The western diet is probably excessive in total intake as well as in expensive forms of protein like red meat and dairy products. The diets of many Asians are heavily dependent on cereals, but are deficient in vegetables, legumes, and other sources of protein (Table 7).

A factor frequently overlooked is the substantial calorie supply some legumes provide. Table 6 shows that both soybean and mungbean provide calories in amounts equal to rice, but are six times richer in protein and also contain a number of other nutritional elements (AVRDC, 1976). Mungbean is also used for starch and produces large amounts of carbohydrate.

The quality of vegetable protein in human nutrition has been clearly established as equal to protein of animal origin. Recent controversy over the daily protein requirement has resulted in published values ranging from about 1.0 g/kg of body weight (FAO, 1975; WHO, 1973) to values as low as 0.42 g/kg. Given the complexity of protein quality and consideration for protection and growth needs of children, young adults, and lactating mothers; current recommended values should range from 0.75 to 1.0 g/kg (Scrimshaw, 1976).

Vast quantities of peanut, cotton seed, coconut, and soybean meal after oil extraction are currently fed to livestock and poultry, used as organic fertilizer, or exported. Converting this oil meal into attractive, palatable, low cost, high protein, long shelf-life food, particularly for low-income groups, will be a major contribution to the reduction of protein malnutrition in developing Asian countries (Brown, 1974). Some products like Vitasoy, Vitamilk, and soymilk are

Table 5. Protein Productivity of Certain Tropical Food Crops[a]

Crop	Estimated Yield	Protein Content	Amino Acid Score	Crop Duration	Protein Productivity[b]
	kg/ha (1)	% (2)	% (3)	Days (4)	kg/ha Day (5)
Legumes					
(1) Soybean	2,800	38	81	95	9.1
(2) Lima Bean	3,200	25	64	115	4.5
(3) Cowpea	1,800	25	65	80	3.3
(4) Peanut	1,600	26	79	120	2.7
(5) Winged Bean	1,400	31	78	112	3.0
(6) Chick Pea	2,500	20	68	125	2.7
(7) Mungbean	900	24	65	75	1.9
Root Crops					
(1) Sweet Potato	20,000	1.3	63	120	1.4
(2) Potato	15,000	2.0	54	125	1.3
(3) Cassava	20,000	1.2	57	220	0.6
Cereals					
(1) Rice	5,000	7.5	72	140	1.9
(2) Maize	4,000	9.5	49	120	1.6
(3) Sorghum	3,500	10.1	47	110	1.5

a) Luse, R.A. and P.E. Okwuraiwe, 1975. Role of legumes in tropical nutrition. pages 98-100. IN: Proc. IITA collaborators' meeting on grain legume improvement. R.A. Luse and K.O. Rachie, eds. IITA, Ibadan, Nigeria.

b) $\frac{1x2x3}{4} = 5$

popular beverages in HongKong, Singapore, Thailand, India, and Malaysia (Brown, 1974). Other products such as "Nutrinuggets" are meat-like foods made from soybean protein which are fast becoming popular especially among vegetarians in India (Trikha and Nave, 1976).

There is growing interest and awareness regarding legumes as a major protein source in the diet in South and Southeast Asia. Additional resources for intensive research and vigorous extension are urgently needed to take advantage of this opportunity.

The Future Role of Legumes

The improvement of legume production in South and Southeast Asia, like most agricultural development programs, demands a complex set of thrusts. Combined attacks of an applied research and efficient extension system are necessary (Lumpaopong, 1976), which will offer solutions to

Table 6. Nutritional contribution of AVRDC's six crops towards balancing a rice diet.[a]

Crop (per 100 g)	% H_2O	Energy (Cal)	Protein (g)	Iron (mg)	Calcium (mg)	Vitamin A I.U.	Vitamin B_1 (mg)	Vitamin B_2 (mg)	Niacin (mg)	Vitamin C (mg)
Rice	13	354	6.5	0.6	15	0	0.11	0.04	1.4	0
Soybean	8	325	36.8	7.4	216	20	0.44	0.31	3.2	0
Mungbean	11	320	22.9	4.9	86	70	0.52	0.29	3.1	0
Sweet potato[b]	70	113	2.3	1.0	46	7,100	0.08	0.05	0.9	20
White potato	67	75	2.3	0.7	7	0	0.07	0.04	1.0	7
Tomato	95	18	0.7	0.4	11	260	0.04	0.03	0.4	29
Chinese cabbage	95	15	1.9	0.7	38	+	0.10	0.10	0.4	35
Recommended Nutritional balance[c]		354	7.8	2.4	83	690	0.19	0.20	2.4	7

[a]Adapted from T.C. Tung, P.C. Huang, H.C. Li, and H.L. Chen. Composition of foods used in Taiwan. Journal of the Formosan Medical Association, Vol. 60(11) pp. 973-1005, Nov. 1961. [b]Orange-fleshed sweet potato roots. [c]Combined averages of recommended balance when caloric level is 354 Cal (100 g of rice) for adult men doing moderate work and 10-year-old children in Taiwan and India. "+" = Trace.

Table 7. Comparison of per capita consumption of food categories by Asians and Westerners (Oyer, 1976)

Food Category	Asian (Philippines)	Western (American)
Cereals	144	71
Vegetables including legumes and potatoes	67	146
Fruits	53	95
Seafood	35	6
Red meat	19	85
Dairy products	12	129
Poultry	7	23
Eggs	5	14
Total	342	569

some basic scientific problems and will also produce changes in economic and social systems.

Legume improvement programs for the tropics consist of two major approaches (1) adaptation of temperate legumes to the tropical environments and (2) upgrading the yield potential and quality of tropical species. Soybean research is an example of the former and current research efforts on mungbean, winged bean, cowpea, or ipil ipil, of the latter.

The initial critical step in each approach is to identify the production constraints at the farm level and an understanding of the limiting factors and the restrictive problems, and then to provide a research approach that will solve the problems or else elicit information that proves them insolvable with present techniques.

The approach used by four of the international agricultural research centers with legume programs (Asian Vegetable Research and Development Center (AVRDC), Centro Internacional de Agricultura Tropical (CIAT) International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), and International Institute of Tropical Agriculture (IITA) is essentially the same.

1. Assemble the largest possible germplasm collection and develop a massive breeding program.
2. Identify resistance to the problem pest and disease species and use the resistant materials in the breeding program.
3. Seek physiological reasons for tolerances to heat, moisture, sunlight, and soil factors, and changes in plant architecture.
4. Overcome management limitations in the field.
5. Attempt improvement or, at least, retention of quality factors, both nutritional and organoleptic.

An excellent recent statement of objectives for a legume improvement program (Rachie and Rawal, 1976) points out the urgency of rapid progress and the necessity to establish both long and short range goals. Research programs must be directed toward practical solutions.

Research priorities have been established for several legume research programs at International Agricultural Research Centers. Some examples are given below.

Soybean research in Asia (AVRDC, 1976) has the following priorities:

1. Increase the yield potential and reduce sensitivity to photoperiod in humid tropical environments.
2. Incorporate resistance to major soybean diseases such as soybean rust *(Phakopsora pachyrhizi)*, bacterial pustule *(Xanthomonas phaseoli* var *Sojensis)*, purple seed stain *(Cercospora kikuchii)*, downy mildew *(Peronospora manshurica)*, soybean mosaic virus, and nematodes.
3. Develop tolerance to beanfly *(Melanagromyza* sp.*)* and other insect pests.
4. Improve our understanding of nitrogen fixation, transfer, and plant metabolism in relation to seed yield and plant growth.
5. Improve field management practices such as fertilizer efficiency, weed control, irrigation, and multiple cropping.

Mungbean research priorities have been identified as follows (AVRDC, 1976):

1. Improvement of yield potential and plant architecture and produce stable yields with few harvests (determinant growth).
2. Improve mungbean resistance to Cercospora leaf spot *(Cercospora canescens)*, downy mildew, and root diseases *(Pythium* sp., *Rhizoctonia* sp. and *Fusarium* sp.).
3. Incorporate resistance to the beanfly *(Melanagromyza phaseoli)* and the southern cowpea weevil *(Callosobruchus chinensis)*.
4. Improve field management practices and the economics of crop production.

Pigeon pea *(Cajanus cajan)* improvement objectives are stated below (ICRISAT, 1975):

1. To obtain materials with high yield potential.
2. To ensure stability of yield through disease and pest resistance as well as drought tolerance.
3. To develop improved nutritional quality and consumer acceptability.

A chickpea *(Cicer arietinum)* breeding strategy also has been developed for the semi arid tropics (ICRISAT, 1976):

1. Breed and generate plant materials with high yield potential, stability, and wide adaptability with due emphasis on quality considerations.
2. Find resistance to the "Wilt Complex" and *Ascochyts* blight.
3. Improve plant competition capacity especially for water and nutrients.

Peanut *(Arachis hypogaea)* research also is being organized at

ICRISAT (1976) and is drawing on many other significant programs to accelerate research with the following objectives:

1. Resistance or immunity to disease through genetic improvement (especially rust, *Cercospora*, rosette, etc.).
2. Resistance to *Aspergillus flavus* infection of seed.
3. Study the physiology of drought resistance or avoidance.
4. Microbiological studies of nodulation and nitrogen supply to the crop.

Other major legume improvement programs have been initiated to non-Asian locations, which may directly contribute to improvements in these species in Asia. IITA (1976) has a major program with similar objectives for the cowpea *(Vigna unguiculata)*, and lower priority attached to soybean, lima bean *(Phaseolus lunatus)*, pigeon pea, winged bean *(Psophocarpus tetragonolobus)*, African yam bean *(Sphenostylis stenocarpa)*, and velvet bean *(Mucuna pruriens* var. *utilis)*.

CIAT (1976) in Colombia has an intensive research program on field or dry beans *(Phaseolus* sp.*)*.

Progress made so far at AVRDC, INTSOY, IITA, ICRISAT, and CIAT clearly indicate the potential to increase legume production in South and Southeast Asia.

Several soybean varieties from the temperate countries and selected accessions from the germplasm collection have shown a yield potential of about 4 t/ha in about 100 days in tropical Asia (AVRDC, 1975, 1976; Shanmugasundaram, 1976_c). Photoperiod insensitive lines have also been found in the soybean germplasm collection. Based on the photoperiod response and adaptability to different times of planting, a tropical classification system for soybean has been proposed (Shanmugasundaram, 1976_b).

Mungbean varieties have been identified with a yield potential of 2.5 t/ha in about 80 days (AVRDC, 1975). For both soybean and mungbean, sources of resistance to major diseases have been identified, and these sources are being combined with high yielding lines. Similar results have been obtained with other food legumes at IITA, ICRISAT, and CIAT.

All these results indicate the potential to increase the food legume production in Asia through the understanding of production constraints. Research to solve or minimize production risks through breeding and management so that legumes are profitable for farmers to produce and less expensive for the consumer is underway. The serious desire of the international community to achieve nutritionally sufficient diets for all can become a reality only with the willing cooperation and active participation of the countries involved.

All the international centers will develop new, high-yielding, improved Food legume varieties and they will also make available suitable cultural practices. These must be linked to an aggressive, national crop production scheme to increase production sharply. "Before the production campaign is launched, a realistic policy of grain pricing must be established and guaranteed by governments to stimulate the farmer and to assure him a fair return for his investment." (Borlaug, 1975). Food legumes have a major role in bringing about a global "protein revolution" in order to save millions from protein malnutrition and its consequences.

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THE ROLE OF LEGUMES IN TROPICAL AMERICA

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INTRODUCTION

Tropical America has contributed a large share of the world's legume diversity. Many of the most valuable legume species are native to the region. Cultivated and wild forms abound in its farming systems. Socially and economically legumes are very important to Tropical America. They are used in many ways and fuel the region's cash flows.

This paper focuses on the role of legumes in Tropical America's farming systems. It covers mostly typical tropical areas of Latin America, including the Caribbean zone but excluding Argentina, Chile, Paraguay, and Uruguay (Pinchinat, *et al.*, 1976). By necessity only a sample of different legume groups is handled here.

TYPES

Many food legumes as defined by Roberts (1970), are grown in Tropical America but some have only local value. The most commonly cultivated types in the region are common bean *(Phaseolus vulgaris)*, coastal bean *(Vigna sinensis)*, lima bean *(Phaseolus lunatus)*, pigeon pea *(Cajanus cajan)*, dolichos bean *(Dolichos lablab)*, tepary bean *(Phaseolus acutifolius)*, adzuki bean *(Phaseolus angularis)*, mung bean *(Vigna radiata)*, and scarlet runner bean *(Phaseolus coccineus)*. Peanut *(Arachis hypogaea)* is also grown and lesser food legumes such as the American yam bean *(Pachyrrhizus tuberosus* and *P. erosus)* and asparagus bean *(Vigna sesquipedalis)* may be seen. In recent years efforts have been stepped up to introduce the winged bean *(Psophocarpus tetragonolobus)* into the region's farming systems. The common, lima, runner, tepary, and American yam beans, as well as peanut, figure among the agriculturally important food legumes that are native to Tropical America. Cubas bean *(Phaseolus coccineus darwinianus)* is also reported to be a natural interspecific hybrid between *P. coccineus* and *P. vulgaris*, originated from the Sierra Madre Oriental part of Mexico (Miranda, 1968).

Forage legumes may be conspicuous in the region's natural grasslands but normally they are not purposely cultivated, except on a limited scale. Some of the most frequent species belong to the genera *Desmodium, Aeschynomene, Phaseolus, Centrosema, Indigofera, Stylosanthes,*

Pueraria, Alysicarpus, Calopogonium, Clitoria, Stilozobium, Eriosema, Rhynchosia, and *Canavalia*. A random sample of native forage legumes may include tick clover *(Desmodium canum)*, stylo *(Stylosanthes guyanensis)*, Centro *(Centrosema pubescens)*, jack bean *(Canavalia ensiformis)*, calopo *(Calopogonium mucunoides)*, and the streaky bean *(Clitoria ternatea)*.

A large number of legume trees grows practically wild in Tropical America. Among the most common are tamarindo *(Tamarindus indica)*, white propinac *(Leucaena glauca)*, guanacaste *(Enterolobium cyclocarpum)*, cockspurs *(Acacia* sp.), sennas *(Cassia* sp.), mesquites *(Prosopis* sp.), corals *(Erythrina* sp), mother-of-cacao *(Gliricidia sepium)*, saman *(Pithecolobium saman)*, and the various guamas *(Inga edulis, I. vera, I. laurina, I. spectabilis* and others). Many well known species such as timbo *(Lonchocarpus nicou)*, tonka *(Dipterix odorata)*, guapinol *(Hymenaea courbaril)*, along with white propinac, the guamas, and saman are of Tropical American origin.

This partial list illustrates the impressive diversity of the legume resources of the region. More comprehensive treatments of the subject are offered by Standley and Steyermark (1946), Whyte *et al.*,(1953), Mateo Box (1961), León (1968), Oakes (1970), and Lotero *et al.*,(1971), among others.

USES

In Tropical America legumes serve extremely varied purposes, differing from species to species and within the same species. Chief usages broadly fall into two main categories: food and non-food. As food sources legumes are useful to both humans and animals.

Human Food

Socially at least, the direct or indirect utilization of legumes as human food plays a vital role in Tropical America. Because of their protein contents (from about 20% to over 40%) and through combination with other foods (especially cereals), legumes constitute a cheap source of high-value dietary protein. This is a real advantage for the low-income groups, which are in fact the largest social class and major consumers of food legumes in the region.

The amount of legume plant parts (principally dry or green seed, whole green pod, leaves, and fleshy roots) eaten *per capita*, is unusually high in Tropical America. Legume grain intake as shown by dietary surveys in the region may be as high as 89 g/person/day (33 Kg/person/year), according to Bressani (1973). From data tabulated by Jaffé (1971), the contribution of legumes as protein sources in the usual diet of Tropical America (represented by Mexico, Venezuela, and Brazil) amounts to 20 times that in temperate countries (such as Argentina or Sweden).

Regionwide the common bean outranks the other food legumes in consumption. According to Scobie *et al.*, (1974) common bean protein intake in Brazil (14.5 g/capita/day) and Nicaragua (13.5 g/capita/day) represents at least 20% of the daily total protein supply *per capita*.

Estimations from seven selected areas of Tropical America (Gutiérrez *et al.*, 1975) set the lowest caput/year consumption of common bean at about 5 Kg in the Republic of Panama and the highest at 25 Kg in Brazil. But while in Brazil common bean is by far the most consumed pulse, in Panama it significantly shares food importance with other pulses such as pigeon pea and coastal bean.

In general pulse consumption tends to be higher in rural areas and the marginal social classes than in urban and elite classes. Yet in places like Costa Rica, Haiti, Brazil, and Jamaica they are an integral part of the food folklore, appealing to all groups. For example, in Costa Rica, the typical "Gallo Pinto" (common dry bean and rice, re-fried) is a countrywide breakfast meal, and all over Brazil saturday means "feijoada" (common dry bean soup and meat).

In Tropical America many delicious dishes are prepared with mature (but not dry) seed (green beans) of grain legumes such as pigeon pea, coastal bean, common bean, runner bean, lima bean, and dolichos bean. Like the green pea *(Pisum sativum)* of temperate zones, the green seed of pigeon pea is canned in countries such as Puerto Rico, Trinidad-Tobago, and the Dominican Republic. Sprouted beans, the immature pods (string or snap beans), and the leaves of many legumes are consumed as vegetables or in salads.

The consumption of fleshy roots of legumes such as the American yam beans and runner bean in some areas advantageously substitutes or alternates with that of starchy roots such as cassava *(Manihot esculenta)*.

Peanut and soybean by and large are valued more for their oil (and residual meal) than for their other food uses, but in Tropical America their consumption as pulses is sizeable or growing. Peanut is eaten roasted or as butter spread. In Haiti, peanut butter on cassave (a flat cassava bread) topped with watercress *(Nasturtium officinale)* is a favorite snack. The direct use of soybean, as whole seed or flour, is being promoted in countries such as Costa Rica (Pinchinat *et al.*, 1975).

Legumes are consumed in many other ways which have received only scant publicity. Thus the pulp of some legume pods as in tamarindo is utilized to prepare refreshing beverages, and the sweet aril of the seed of the guamas is chewed like candy. According to Martin and Ruberté (1975), the flowers of the streaky bean are cooked with rice to color it.

Feed

Legumes rank among the principal sources of protein for animals, either as forages or as components of concentrated feeds.

The flora of Tropical America is rich in valuable forage legumes. Very useful species are found among the genera *Desmodium, Pueraria, Alysicarpus, Calopogonium, Indigofera, Stylosanthes, Stilozobium, Centrosema, Phaseolus, Clitoria, Aeschynomene,* and many others. But in typical areas of Tropical America the utilization of forage legumes has been almost incidental. Grazing management systems to stimulate legumes in pastures have been glaringly lacking. The foliage of some legume trees (e.g. *Erythrina* sp.) is cut and fed to animals. In the

drier areas many leguminous trees *(Prosopis* and others) are browsed, particularly by goats.

Soybean and peanut meals are principal legume ingredients found in concentrated feeds. In Tropical America the bulk of the meal goes into the preparation of concentrates for poultry, swine, and dairy cattle. Cull bean of some pulses, such as coastal bean, are sometimes utilized to fatten pigs. The seed of mesquites is considered in itself a good concentrate feed for farm animals.

Other Uses

The use of legumes as shade trees is a common practice in plantations of crops such as coffe *(coffea arabica)* and cacao *(Theobroma cacao)*. In Tropical America that purpose is fulfilled with a large array of species among which stand out the corals *(Erythrina poepiggiana, E. Glauca,* and *E. berteroana)*, mother-of-cacao, the guamas, and saman.

As a rule legumes are not intentionally planted in the region to condition the soil. Plant residues from the shade and forage legumes normally help in improving the physical and chemical properties of the soils, especially their nitrogen economy. This aspect however has not received due attention, although scattered efforts have been undertaken to exploit the legume - *Rhizobium* symbiosis.

Apart from edible oil processing, legumes in the region have many other agro-industrial applications. Balsam is obtained from the balsam tree *(Myroxylon balsamum)*, dye from logwood *(Haematoxylon campechianum)*, and tanning from dividivi *(Caesalpinia coriaria)*. The roots of timbo are utilized to produce an insecticide (rotenone) and the seed of tonka, to extract a coumarin-based aromatic essence (Tonka oil). The pods of some species such as saman are used sometimes to prepare alcohol. Many legume trees, including rosewood *(Dalbergia* sp), saman, cristobal *(Platymiscium* sp) and timbo are good sources of timber.

Legumes in Tropical America also have their place in folk medicine (Standley and Steyermark, 1946). Morton (1976) reported some of the medicinal applications of pigeon pea in the Netherlands Antilles, Cuba, and Colombia.

In aesthetic value many of the legumes that grow in Tropical America can hardly be surpassed. The seeds of several corals are used to make bracelets and other decorative articles. The spectacular beauty of Poorman's Orchid *(Bauhinia variegata)* and the flamboyant *(Delonix regia)*, among others, is a priceless gift to the eye.

ECONOMIC VALUE

Production statistics do not cover all the legumes utilized in Tropical America. Many species participate only on a very limited or practically insignificant scale in the trade channels. Readily available statistical information concerns mostly the pulses (including soybean and peanut), stringbeans, and greenbeans. A good portion of the production data presented herein is based on statistics from FAO (1975).

Table 1. Common Dry Bean Production in Tropical America. 1974*

Zone and Country	Harvested area (1000 ha)	Production (1000 MT)
North Central	1932	1203
Costa Rica	22	10
Cuba	35	24
Dominican Rep.	32	31
El Salvador	45	32
Guatemala	110	78
Haiti	41	43
Honduras	67	32
Mexico	1500	896
Nicaragua	64	52
Panama	12	3
Puerto Rico	4	2
South	3844	2370
Bolivia	9	4
Brazil	3500	2168
Colombia	105	73
Ecuador	69	38
Peru	66	50
Venezuela	95	37
T O T A L	5776	3573

* From FAO (1975

Table 2. Pigeon Pea Production in Tropical America. 1974*

Zone and Country	Harvested Area (1000 ha)	Production (1000 MT)
North Central	28	41
Dominican Rep.	13	29
Haiti	7	4
Panama	2	2
Puerto Rico	3	3
Trinidad T.	3	3
South	9	4
Venezuela	9	4
T O T A L	37	45

* From FAO (1975)

Table 3. Soybean Production in Tropical America. 1974*

Zone and Country	Area (1000 ha)		Production (1000 MT)	
North Central	245		410	
Mexico		245		410
South	4875		7660	
Bolivia		2		2
Brazil		4793		7500
Colombia		78		156
Ecuador		1		1
Peru		1		1
T O T A L	5120		8070	

* From FAO (1975)

Table 4. Production of Peanut in Shell in Tropical America. 1974*

Zone and Country	Area (1000 ha)		Production (1000 MT)	
North Central	155		157	
Cuba		15		15
Dominican Rep.		85		75
Haiti		4		2
Mexico		51		65
South	392		519	
Bolivia		7		10
Brazil		350		479
Colombia		1		1
Ecuador		12		11
Peru		2		1
Venezuela		20		17
T O T A L	547		676	

* From FAO (1975)

In 1974 about 6.7 million hectares of pulses (excluding soybean and peanut) were grown in Tropical America, producing nearly 4.3 million metric tons of dry grain. In these totals, the common dry bean amounted to over 86% of the area and almost 93% of the harvested grain. In turn the leading common dry bean producing countries (Table 1) were

Brazil (61% of area and production) and Mexico (26% of area and 25% of production). The Dominican Republic and Venezuela led in the production of pigeon peas (Table 2), one of the most important pulses in the Caribbean sector of the region. Production data for coastal bean are often compounded with those for common bean, thus masking the true importance of coastal bean in Tropical America's farming systems.

Over eight million metric tons of soybean were produced in nearly five million hectares (Table 3), with Brazil accounting for about 94% of the area and 93% of the harvested grain. Approximately 0.7 million metric tons of peanut in shell came from about 0.6 million harvested hectares (Table 4), with Brazil again leading (64% of area and 71% of nut weight).

During 1974, small quantities of string beans were produced, principally in Peru (5,000 metric tons in 1,000 ha) and Venezuela (4,0000 metric tons in 1,0000 ha). The production of greenbeans in the region (Table 5) amounted to 43,000 metric tons (data on harvested area are incomplete).

In local markets, variable but generally small quantities of other edible legumes are traded. In this case as in that of non-food legumes production data are scarce.

The economic worth of legume production is similarly difficult to appreciate in its true dimension because of paucity of reliable statistical information. For common bean some illustrative figures are given by Gutiérrez *et al.*,(1975), showing for instance that this crop brought close to 10 million US dollars to the economy of Peru in 1971 and occupied fourth rank in the agricultural national product of Guatemala in 1964/1975 (Table 6). The sale prices of legume products have reached unexpected high marks. In Costa Rica, for example, the producer currently receives about US$26 for 45 Kg (i.e. nearly 60 cents/Kg) of common dry bean, equivalent to more than three times the price he was paid around the years 1969-70.

CROPPING SYSTEMS

In Tropical America legumes are grown in various cropping systems. Documented information on the subject covers mainly the food legumes and more particularly pulses (including soybean and peanuts).

Common bean is produced mostly on small-size plots which often do not exceed 7 ha (Table 7), although in a few instances, such as in the Cauca Valley (Colombia) and the Dominican Republic, larger units (> 30 ha) can be found (Gutiérrez *et al.*, 1975). Industrial legumes such as soybean tend to be produced in units larger than those for common bean and similar pulses.

Legumes in small farm units in Tropical America are frequently grown in some form of mixed cropping (amorphous polycropping, intercropping, or relay cropping). In larger plantations, pure cropping (mainly in rotation) often prevails. Monocropping is relatively rare. In common bean the proportion of national production that comes from

Table 5. Green Beans Production in Tropical America. 1974*

Zone and Country	Harvested Area (1000 ha)		Production (1000 MT)	
North Central	--		19	
Guadeloupe		--		3
Martinique		--		1
Mexico		8		15
South	16		24	
Ecuador		6		3
Peru		10		21
T O T A L	--		43	

* From FAO (1975)

Table 6. Economic Worth and Ranking of Common Bean in Four Selected Countries of Tropical America 1/

Country	Year	Value (Million US $)	ANP* Rank
El Salvador	1970	5.4	7
Guatemala	1964-65	6.4	4
Nicaragua	1968-70	---	6
Peru	1971	9.5	-

1/ From Gutiérrez *et al.*, (1975)
* Agricultural National Product

Table 7. Size Samples of Common Bean Farming Units in Four Selected Countries of Tropical America 1/

Country	Unit Size (ha)	% of Total Number of Units
Colombia	< 7	50
El Salvador	< 5	96
Haiti	< 1*	--
Panama	< 3*	--

1/ From Gutiérrez *et al.*, (1975)
* National Average

Table 8. Proportion of Common Bean Production from intercropping Systems in Six Selected Countries of Tropical America

Country	Reference*	% of Total Production
Brazil	1,2	80
Colombia	1	90
	2	85
El Salvador	2	50
Guatemala	1	73
Honduras	1	5
Mexico	2	58

* 1 = Gutiérrez *et al.*, (1975) 2 = Scobie *et al.*, (1974)

intercropping alone may vary from as low as 5% in Honduras to as high as 90% in Colombia, according to data compiled by Scobie *et al.*, (1975) for six selected countries of Tropical America (Table 8). The farmers who mix crop also tend to adopt low-cost levels of technology (Bazán, 1974). Typically the farmers use their own seed, apply unsufficient amounts to fertilizers and pesticides, and depend almost exclusively on hand labor for all farming operations. Poor seed quality (Sánchez and Pinchinat, 1974), low planting densities, low levels of soil fertility, deficient weed and insect management, and poor protection against diseases are in a large measure responsible for the low legume yields observed in the region, especially in common bean (618 Kg/ha). Nevertheless although apparent yields for Tropical America may be smaller than those from other regions, in such comparison the cropping systems involved must be taken into consideration. Normally food production in intercropping and other forms of polyculture is more efficient than in monocropping (Pinchinat *et al.*, 1976).

CONCLUDING REMARKS

Legumes play a vital role in Tropical America's agriculture. Yet although practically all the countries of the region have had some kind of legume program, its scope in most cases has been at best narrow and its structure weak (Pinchinat, 1973). Some notable exceptions have been Colombia and Brazil in South America, and El Salvador and Guatemala in Central America.

From the beginning of this decade marked food shortages, especially animal proteins, have stirred renewed interest in legumes. A strong case of food legume research and production was presented by Roberts (1970). Thereafter many stimulating events took place in the region, promoting legume production and technical support.

In the early 1970's the Central American Cooperative Program for the Improvements of Food Crops, better known as PCCMCA for its initials in

Spanish, added coastal bean and soybean to its original common bean project (Miranda, 1972); by 1974 it had also begun research in pigeon peas.

In a seminar organized by the Institute of Nutrition of Central America and Panama (INCAP) in 1971 in Guatemala, on protein resources in Latin America, Jaffé (1971) praised the importance of grain legumes as protein sources for that part of the world. In that same year Brazil's bean work was reviewed in the first Brazilian bean symposium, held in Campinas, Sao Paulo (Brazil, Min. Agr., 1972).

An International bean program based in Colombia was established in 1972 under the responsibility of the Centro Internacional de Agricultura Tropical (CIAT, 1975). A year later CIAT sponsored in Palmira, Colombia an international seminar to evaluate the potential of common bean and other food legumes in Latin America (CIAT, 1973). In connection with that seminar Pinchinat (1973) carried out a survey concerning the situation of food legumes in Latin America up to the period 1972-1973. An enlarged treatment of the information gathered through the survey was prepared by Gutiérrez *et al.*, (1975).

The nutritional aspects of common bean and other grain legumes as animal and human foods were discussed in a seminar that took place in Riberão Preto, Minas Gerais, Brazil, late in 1973 (Jaffé, 1975). A workshop on tropical diseases of legumes, with special attention to Tropical America, was held in Puerto Rico in 1974 (Bird and Maramorosch, 1975). At the end of that same year legume yield constraints, again with emphasis in Tropical America, were analyzed in a symposium on nutrition and agricultural development in the tropics organized by INCAP in Guatemala (Scrimshae, 1976). Numerous other meetings have been held and other types of activities undertaken, mostly on local scale, to strengthen national legume programs in the region.

Given the attractive prices now paid for legumes and legume products, and the food customs and agricultural traditions in Tropical America, legumes are assured a first class seat in the region's agriculture. They deserve it.

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SOME MAJOR FERTILITY PROBLEMS OF TROPICAL SOILS

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INTRODUCTION

Much has been written about the infertility of tropical soils - frequently with the implication that the tropics have problems which are encountered nowhere else. It is probably safe to say that there is nothing unique about the type of soil fertility problems encountered in the tropics. Nutritional problems are formidable obstacles to production and toxicities associated with soil acidity are frequently encountered in all climatic zones and not just in the tropics.

Neither can it be said that problems are more intense in the tropics. It is difficult to imagine, for example, a more intense nitrogen deficiency than sod-bound brome grass in the North Central USA or phosphorus deficiencies which exceeds those encountered in some calcareous soils of the semi-arid West.

Soil fertility problems are not necessarily more complex either, although multiple deficiencies tend to be the rule rather than the exception for leached and weathered soils in the humid tropics.

What is more unique about the tropics is the magnitude of soil fertility problems in relation to resources which are being committed or are being advocated, for alleviating those problems.

SOME PHILOSOPHICAL PROBLEMS

The fundamental philosophy of research, extension and practice poses, in many instances, major limitations in managing the fertility of tropical soils. We will list as examples three concepts which to some extent prevail in professional circles in the tropics and also influence decision making about agricultural production. These are:

1. The majority of subsistence farmers cannot afford cash inputs and even if they could the necessary infrastructure is usually not established to provide their needs or utilize their products.
2. The first increment of an input gives the largest increment of yield --which justifies emphasis on a program of minimum inputs.

3. What we need is a new variety, or better still, a new crop, which can thrive on soil infertility.

The above concepts need some revision before substantial progress can be made for sustained increased production in most tropical areas. Our opinion concerning these concepts follow:

First, It is often assumed that in tropical systems of crop production, as compared with agriculture in developed countries, fertilizers are expensive and that crop produce is relatively cheap. Therefore, much higher crop responses per unit of nutrient are normally needed in the tropics if fertilizers are to be profitable. But this conclusion is true only if the assumptions are valid; and it is not always correct to assume that crop produce in the tropics is always cheap either on a relative basis or in absolute terms.

The philosophy that subsistence farmers cannot afford sophisticated technology has its element of truth, of course. But we must not forget that farmers in technologically developed countries did not evolve, with their own resources, the practices they now use so effectively. Fertilizers have usually been heavily subsidized by one means or another in the technologically advanced countries. We may reasonably suppose that even more subsidies will be required in those areas of the tropics where population pressures or declining soil fertility have already brought agriculture to subsistence levels. Neither must we insist that slow recovery rates of added fertilizer are altogether bad. Chaminade (1968) has reminded us that the quantities of fertilizer needed to increase the fertility of soils depends essentially on soil properties. This increase of fertility must be considered a capital investment which will be paid off, although perhaps not before ten years or more. It cannot possibly fail to be of future help in developing countries and perhaps is the most efficient form of help that can be given them. This is especially true for nutrients which are efficiently recycled in the cropping system.

Subsistence agriculture in much of the Tropical Zone already is operating against a soil fertility constraint. It cannot generate the capital required for investment in long range fertility building; and, without fertility inputs there is little likelihood of sustained, increased production.

If we may coin a proverb: "An 'Operations Bootstraps' cannot succeed in a land where there are no shoes." Or, as Dr. Uehara has already said in these proceedings "--a poor farmer on infertile land has little hope of extricating himself from poverty's grip."

Richardson (1968) reports that the Freedom from Hunger campaign has shown, in West Africa at least, that fertilizers even used alone can be more effective on traditional farms than had formerly been thought. The financial return on the fertilizer bill can be very substantial. Starting with fertilizers alone, other methods of agricultural improvement will follow which will then lead to even greater benefits from the fertilizer. For this reason FAO regards fertilizers as "The spearhead of agricultural development."

Second, it is assumed by some agricultural economists that the Mitcherlich yield response curve is always valid at low rates of fertilization--that is, that the curve is concave downward throughout its entire range. That such is not always the case can be demonstrated whenever the soil, or microorganisms, or weeds, compete strongly for the first increment of nutrient applied.

What is desperately needed are crop yield response curves covering the most important nutritional problems. The experimental work should meet the following specifications:

1. Rates of fertilizer cover the range from zero applied to excess.
2. Enough experimental points to define the yield response curve throughout its range.
3. Agronomic practices provide possibilities for acceptable yields.
4. Experimental treatments not confounded by other variables.
5. A field plot design which provides some replication and a degree of randomization.
6. Relevant collateral information collected, especially on soil and weather.
7. Experiments continued for sufficient time so that residual effects can be evaluated.

It is amazing how little information there is of this kind in the tropics or anywhere else. Unfortunately too much effort has been spent on documenting beyond reason that a nutrient is, or is not, deficient and on working out interactions which have been well documented before.

Most agriculture in Northern Europe and Eastern North America could not remain competitive without a large input of plant nutrients and soil amendments - high yielding varieties and all other technology notwithstanding. And yet the prevailing philosophy of agricultural production in the tropics is one of minimum inputs. Typically this resolves to a system of no inputs of fertilizer or lime. Since extensive areas in the tropics are so low in plant nutrients that continuous cropping without fertilizers is impractical, it is clear that radical upgrading of production will require the development of new systems of agriculture based on the intensive use of fertilizers and improved crop varieties which will respond to the higher level of fertility and accompanied by improved systems of soil and crop management (Russell, 1968).

As for item three, - the prospects for crops which will succeed on infertile soils -we are treading on hallowed ground. Yet we need to remind ourselves, at least, that the general history of crop production runs counter to the idea that quality crops can thrive on infertility. Miracle rice, for example, was designed to utilize high fertility. Its successful culture demands more nutrients, not less. It now seems that even cassava, a crop which is noted for its ability to succeed where most crops starve, has a rather large requirement for potassium, phosphorus and zinc and responds to lime as well (Spain *et al.*, 1974). Plant tolerance to toxic concentration of nutrients has been successfully dealt with by plant breeding in a number of instances and more progress will probably be made with that approach. Also it is likely that, through breeding,

plants can be made to scavenge more thoroughly for nutrients. But we must beware lest we begin to believe that through breeding we can accomplish the impossible of utilizing nutrients that aren't there.

There is yet a fourth idea which is popular among administrators and may even seduce research workers as they prepare project proposals. That is, a child-like faith that a major production breakthrough is imminent if we would only put forth enough research effort ..... Well, hope does spring eternal ..... We wouldn't want to live in a world without it. But there is some danger that in searching for the breakthrough we may neglect the mundane things that work for sure. While we seek to make nitrogen fixers out of grasses let us not neglect to inoculate legumes.

SOME LIMITING NUTRIENTS

In the remainder of this exercise we will identify some of the more probable soil fertility constraints in the tropics. Of the numerous probable problems we will deal with only three in any detail - P, S, and Mo deficiencies. Toxicities and deficiencies associated with low soil pH are scarcely mentioned since this important subject is dealt with elsewhere in these proceedings. Finally we will try to show how some of these problems interact to produce a mosaic of variable crop production within a small areas.

Phosphorus Deficiency and Phosphate Fertilizer Requirements.

Phosphorus deficiency certainly is one of the important soil fertility problems of the tropics. This is not to imply that all soils of the tropics are phosphate deficient or even that those which are deficient immobilize much phosphate. Some soils are not deficient, and many that are do not adsorb much phosphate so that immediate phosphate fertilizer requirements are small.

There is abundant evidence that the immediate source of phosphate for plants growing in soils is the soil solution. Numerous studies have demonstrated a close relationship between phosphate in the soil solution or in dilute salt solution equilibrated with the soil, and the phosphate nutrition of plants. The phosphate concentrations of typical soil solutions are usually low. Values of about 0.1 μg P/ml are not uncommon for the bulk solution of fertile soils. In the vicinity of roots, concentrations may be much lower; and in phosphorus deficient soils with which we have worked in Hawaii and Nigeria, P concentrations in equilibrium solutions are often less than 0.01 μg/ml.

For high phosphate flux to roots of plants, phosphate in soil solutions must be renewed quickly and continuously. This renewal mostly involves phosphate desorption from soil mineral colloids unless, as is sometimes the case, organic P is being mineralized rapidly. Phosphate is returned to the adsorbed state when soils are fertilized, or when P is released, from organic or inorganic materials in amounts exceeding plant requirements.

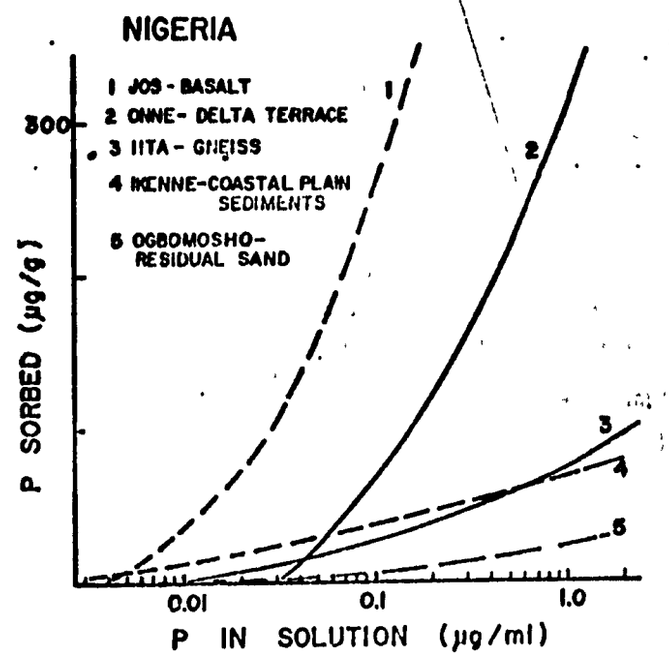

Figure 1. Phosphate adsorption curves for 5 Nigerian soils showing the influence of texture and mineralogy on the buffering capacity of soils for phosphate.

Thus the phosphate sorption-desorption process is an important regulator of the phosphate supply to crops and phosphate requirement of soils.

All factors influencing phosphate adsorption can interact to produce widely ranging phosphate adsorption characteristics of soils. Some examples of phosphate adsorption curves are presented in Fig. 1. From these curves of Nigerian soils it is obvious that the quantity of phosphate required to adjust the amounts of P in the soil solution to some standard concentration 0.2 µg/ml for example (Beckwith, 1964), would be quite different for the various soils. The phosphate requirement by the clay soil developed from basalt was in excess of 350 µg P/g while the loamy sand from Ogbomosho required less than 10 µg P/g. The intercepts of the curves (concentrations) at zero P sorbed (added) give an estimate of the immediate P status of the soils.

Adsorption curves give reasonable estimates of phosphate requirements of soils for crop production if the solution levels required by the crop are known. First approximations of the external P requirements of plants may be obtained from solution culture or sand culture experiments. Asher and Loneragen (1967) have used flowing culture techniques to obtain data on several species. There are some obvious disadvantages of growing plants apart from a soil environment. Phosphate diffusion through soil pores and through water films is different from bulk movement of phosphate in flowing solution cultures.

Perhaps a more important difference between soil culture and solution culture is the likelihood of assistance from mycorrhizal fungi in the soil environment. If such is the case the external P requirement based on nutrient culture data may be an order of magnitude greater than field requirements.

Data on external P requirements of crops should be obtained from

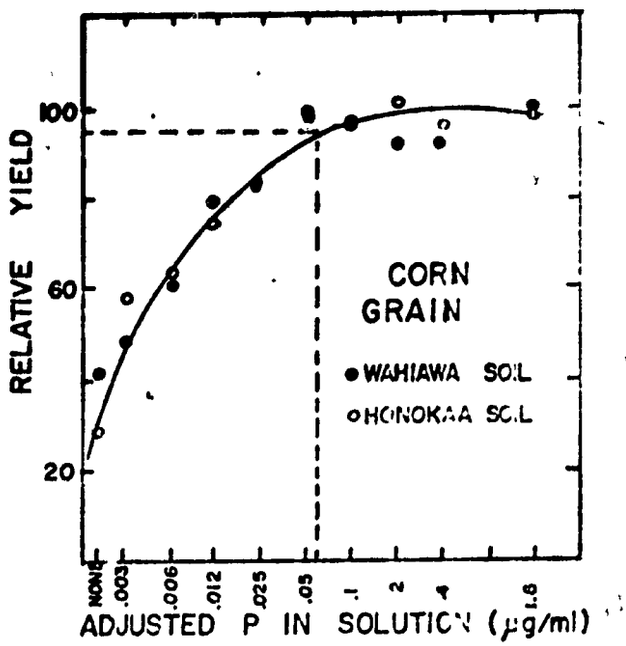

Figure 2. Yield of corn in relation to adjusted concentrations of P in solutions equilibrated with soils having different mineralogical systems. The Wahiawa soil is predominantly Kaolin; the Honokaa soil contains amorphous hydrated oxides of Al and Fe predominantly.

field experiments. Unless special precautions are taken, potted soils are not satisfactory for anything except short term experiments because of the interception of roots by the walls of the pot. This "barrier effect" essentially removes roots from the soil environment and produces a unrealistically high external P requirement (Fox and Kamprath, 1970).

Field experiments which have attempted to measure the requirements of crops for P in soil solutions are rare. Le Mare (1964) found that responses of cotton to phosphate were unlikely if the phosphate potential ($\frac{1}{2}$ pCa + pH_2PO_4) was less than 7.0 but was very probable if it exceeded 7.5. Assuming that calcium in the soil solution is 0.01 M, these phosphate potentials correspond to approximately 0.01 and 0.03 μg P/ml.

During the past five years we have established in Hawaii and Nigeria a range of P concentrations in a series of field plots. Numerous crops have been grown at one or more of these locations. The relative yield response curve, plotted against soil solution concentration gave the quantity of P in solution at specific levels of production. Some of this work has been published (Fox *et al.*, 1975, Nishimoto *et al.*, 1975). The data show that plants differ greatly in their external phosphate requirements. An adequate level for most crops is within the range 0.01 to 0.4 μg P/ml solution (0.01 M $CaCl_2$).

The Hawaii data also suggested that the concentration of P required in solution by corn was independent of phosphate adsorption capacity of soils. Figure 2 demonstrates that a single line described the yield response curves for two soils, which were mineralogically dissimilar. The external P requirement for 95% of maximum yield was

about 0.05 μg P/ml. Fertilizer required to attain 0.05 μg P/ml was much greater for the Honokaa soil than for the Wahiawa soil.

Similar field experiments have been performed on sandy soils at three locations in Nigeria. The yield response curves were relatively flat in all cases and the external P requirement seemed to be directly related to the quantity of light available (Fox and Kang, 1977). For 95% of maximum yield, corn grown in the middle of the rainy season required only 0.01 μg P/ml soil solution, but maximum yield was only 3750 kg/ha while corn grown in an adjacent field, but at an earlier planting date when light intensity was greater, required 0.025 μg P/ml.

Generalizations can be made about the relationship between soil mineralogy and texture and phosphate sorption-desorption. For example, the more silicon depleted and less crystalline soils retain phosphate very strongly. In general phosphate retention by the various mineralogical systems of soils can be ranked as follows: quartz and aluminum-free organic matter < 21:1 type clays < 1:1 type clays < silicon depleted amorphous materials (Fox *et al.*, 1962, Fox and Kamprath, 1971).

Well crystalized clay minerals usually are not extremely reactive toward phosphate. However, Jones and Uehara (1973) demonstrated that the crystalline materials of some soils are coated with amorphous gel-like materials. In highly weathered soils, amorphous material tends to be depleted of silicon (Fox *et al.*, 1971) and there is evidence that amorphous materials immobilize phosphate in relation to the extent they are silicon depleted. Table 1 illustrates this point very well for soils derived from volcanic ash. The principle applies widely in the tropics because volcanic ash is recognized as an important soil forming material in the tropics and ash is probably even more widely distributed than most pedologists realize.

Uehara has said in his contribution to these proceedings that there is a fundamental difference between the highly weathered soils of the tropics and the bulk of the remaining soils of the world. This fundamental difference pertains to surfaces which interact strongly with anions such as phosphate and charge characteristics of those surfaces which bring the reactive surface and phosphate ions together. One possible mechanism by which phosphate ions are held is presented in Fig. 3.

A review by Russell (1968) of field trials performed in Africa has shown that tropical soils there do not generally have high phosphate fixation capacities. He reports that responses by crops to small phosphate fixation are frequently obtained and that residual effects from a single application are reported. This agrees with field research we recently did in Nigeria (Fox and Kang, 1977) in which we were able to detect the effect of two kg/P/ha on the early growth of maize.

Low P requirements of West African soils should be expected. In the first place, they tend to be predominantly quartz sand which does not adsorb phosphate. In the second place amorphous coatings of the type and abundance seen by Jones and Uehara (1973) in Hawaiian soils have not been reported in West Africa.

Phosphate adsorption curves of the highly weathered soils of tropical America (Fox, 1974) and results from field plot results of the

Table 1. Phosphate sorption by recent volcanic ash and soil derived from ashes which had been variously depleted of Si by weathering. Soils from Mt. Irazu and vicinity, Costa Rica. (Data from Fox, 1974)

Soil	Phosphate sorbed[1/] by soil (µg P/ml)	Si in saturation extract of soil (µg Si/ml)
Cinders from crater rim	20	46
Ash from eruption 4 to 6 years previously	20	12
Umbric Vitrandept	425	19
Typic Dystrandept	1900	6
Oxic Dystrandept	2500	2

1/ The supporting electrolyte was 0.1 *M* $CaCl_2$ containing 0.2 µg P/ml after equilibration for 6 days.

kind reported for the cerrado of Brazil (near Brasillia) by the North Carolina State University group (Sanchez, 1974), where the highest maize yields were obtained from the largest quantity of phosphate fertilizer applied (1960 kg P/ha) suggest that the P problem in tropical America is different from West Africa.

Since many soils of the tropics require large amounts of phosphate to make them productive, one major concern is that fertilizer phosphate which is not utilized by the immediate crop may be irretrievably lost through reversion to fixed forms. In some cases this concern is well grounded as in the case of some hydromorphic soils which go through cycles of oxidation and reduction. On the other hand there is abundant evidence of residual effects or carryover benefits to succeeding crops, and in the case of massive phosphate applications, this continues for several years.

An example of short term residual effect is given in Table 2. In this illustration modest phosphate applications were made and two to five crops of maize were grown. There were residual effects because the soil, an Egbeda sandy loam, represented by curve 3 in Figure 1, did not have a high capacity to adsorb phosphate.

Long term residual effects of phosphate fertilizers are not restricted to soils with low capacities to adsorb phosphate. Experimental work in Hawaii (Younge and Plucknett, 1966 and Fox *et al.*, 1968, 1971) demonstrate that residual effects of Phosphate influenced phosphate adsorption curves and the growth of grass-legume sward 11 years after the phosphate was applied (Fig. 4). The soil was a Gibsihumox. It is

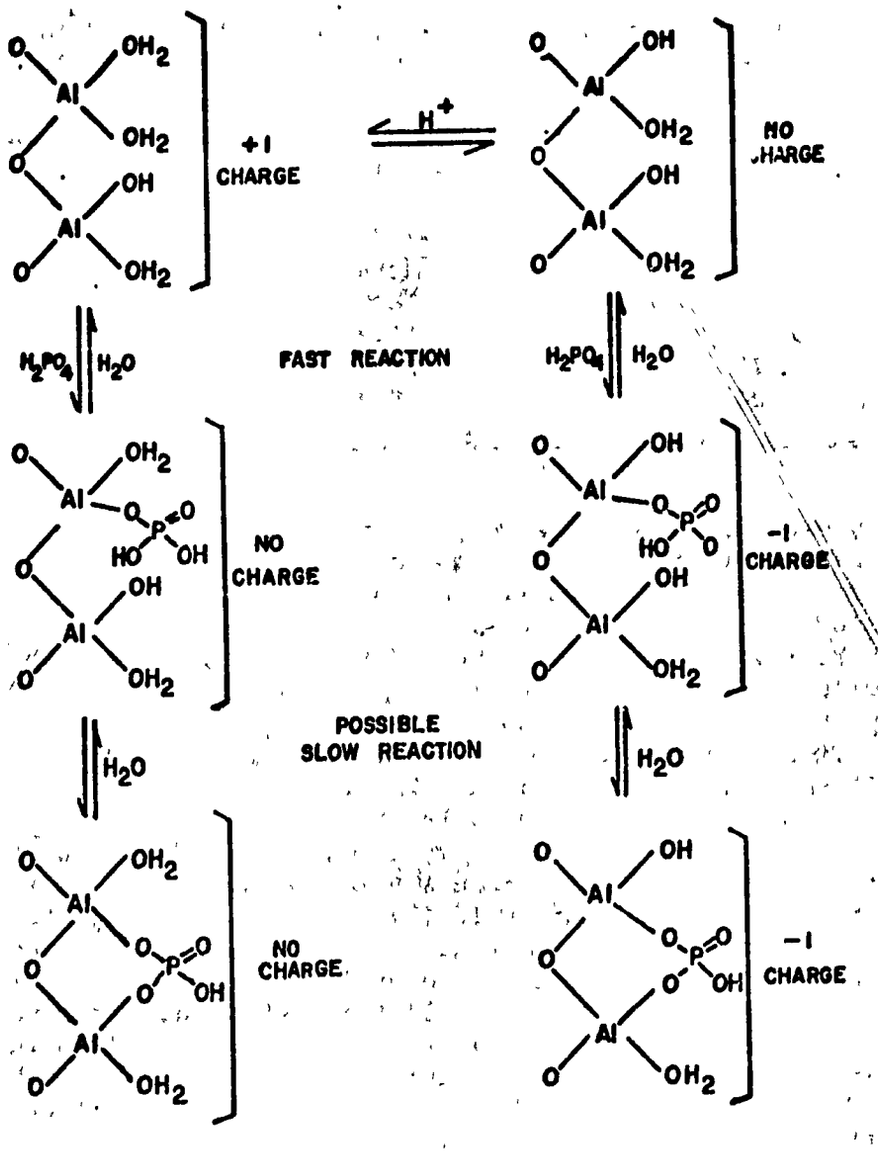

Figure 3. A possible mechanism for phosphate ion retention by highly weathered soil and the slow reversion which continues after the initial reaction.

Table 2. Influence of residual phosphorus on the yield and protein content of soybean grain, Cultivar Bossier. IITA, Ibadan, Nigeria (Data taken from Luse, *et al.*, 1975).

Initial P added	Grain Yield	Grain Protein	
kg/ha	kg/ha	%	kg/ha
0	1355	32.7	479
26	1839	37.5	630
52	2199	40.5	814
78	2248	41.0	842
104	2563	42.5	995

highly weathered and has a very great capacity to adsorb phosphate. Phosphate additions must be made in proportion to the capacity of soils to adsorb phosphate, otherwise notable residual effects could hardly be expected.

Younge and Plucknett (1966) developed the concept that the capacity of soils to fix phosphate could be decreased by phosphate fertilization. Phosphate which is converted to unavailable forms may still be useful if it decreases the phosphate requirement of subsequent rounds of fertilization. That this is a valid concept has been demonstrated by recent experience (Fox *et al.*, 1974).

Soil organic matter may participate in P nutrition of plants in at least two ways. First, inorganic P may be released as a result of mineralization. Jones and Wild (1975) have made some estimates of the phosphate which might be mineralized in the soil of the West African Savanna. If, as they assumed, about 4 per cent of the soil organic matter is mineralized per year, if the soil organic phosphate content is 15 to 60 µg P/g soil, and if the top 30 cm of soil is involved then the amount of phosphate released should be 2.4 to 10 kg P/ha. Not all of this phosphate would be taken up by the crop but at low levels of fertility even this small quantity may be important.

It should be pointed out that correlations between soil organic phosphate and plant uptake of phosphorus have usually been low and non-significant. Probably one of the reasons for inconsistent results is the variable phosphorus composition of organic matter. Singh and Jones (1976) report that the critical concentration of P in organic matter before there is evidence of P release is 0.3%. When organic materials containing less than 0.3% P were decomposed, soil phosphorus was immobilized.

A second effect of organic matter on P nutrition is through its effect on P retention by soils. Humus and simpler organic anions which derive from organic decomposition may become adsorbed onto soil mineral surfaces which would otherwise adsorb phosphate. Also, adsorbed organic matter tends to depress the zero point of charge, the pH at which net

charge on colloidal surfaces become zero. Thus for a given pH, organic matter gives to soil a greater negative charge, which in turn tends to repel anions (including phosphate) from the surface. Presumably, through this mechanism, phosphate has less opportunity to form the stable complexes with inorganic colloids which are characteristic of ligands (Fig. 3).

That these effects probably are important can be supposed from several lines of evidence. In the first place low organic matter-containing subsoils, especially in the tropics, are much more phosphorus deficient than topsoils. Per unit of clay, subsoils almost invariably have larger phosphate adsorption capacities than do topsoils. Singh and Jones (1976) demonstrated a shift in P adsorption curves to the right (decreased P adsorption) as a result of organic matter additions. Fox *et al.*, (1968) observed the effects of adding phosphate to exposed subsoil of a Gibsihumox and subsequently cropping it for several years. In some cases the effect of the phosphate was to decrease subsequent phosphate adsorption more than could be accounted for by the amount of phosphorus remaining. This they believe may have been caused by organic residues which accumulated in proportion to phosphate fertilizer added.

Obviously, low organic matter content is a problem of first magnitude in many tropical soils. The problem is especially severe in the savanna when annual burning is practiced.

The use of organic phosphate as fertilizer has sometimes been advocated as a means of avoiding fertilizer inefficiencies because of phosphate fixation by soils. Advocates of this procedure generally underestimate the long term efficiency of conventional phosphate fertilizers and they overlook the costs of organic phosphates which would certainly greatly exceed inorganic fertilizers.

A more likely approach, which needs detailed attention, is foliar applications of inorganic phosphates. Reports from India claim that grain legumes benefit from combined applications of soil and foliar applied phosphate. Recent research by J.J. Hanway and his associates at Iowa State University indicates that late foliar applications of fertilizers containing NPKS after the onset of blooming may increase soybean yields above what can be attained by soil fertilization alone. It is possible that late foliar fertilization, coupled with localized soil-applied phosphate, might circumvent some of the problems of phosphate inefficiency in soils which immobilize much phosphate.

Sulfur Requirements of Crops in Relation to Sulfur Supplies in the Tropics

Sulfur deficiencies in the tropics have been recognized for many years. The deficiency is particularly widespread in the savannas of Tropical America and Africa. Bolle-Jones (1964) reviewed the early work on sulfur deficiency in Africa. He tentatively concluded that deficiencies are most likely on ferruginous and ferrallitic soils

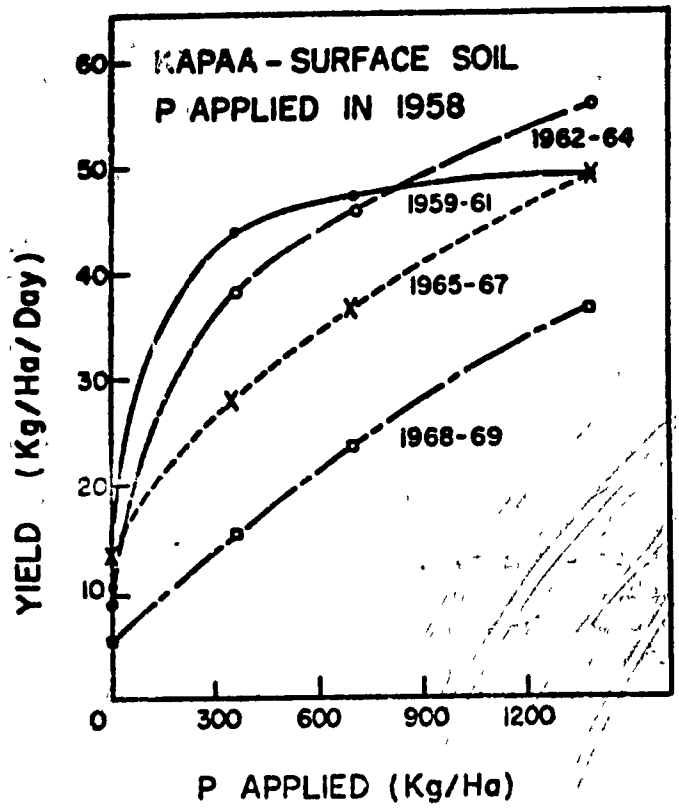

Figure 4. Yields of dry forage (*Digitaria decumbens* plus *Desmodium* species) during 11 years following an application of phosphate fertilizer.

Data from Fox *et al* (1971).

developed on very old erosion surfaces which separate the major drainage systems south of the Sahara. He listed the crops which were good indicators of sulfur deficiency as legumes (groundnuts and lucerne) and certain oil bearing crops, (groundnuts and cotton). Bromfield (1972) has examined sulfate movement in soils of the savanna

Sulfur deficiency was described as a major fertility problem for development of the Brazilian Campos by McClung and Freitas (1959). They interpreted the data they had obtained to indicate that sulfur was second only to phosphorus as a nutritional factor limiting growth of grasses. McClung *et al.*, (1959) believed that responses to sulfur in central Brazil would not be common unless N and P levels were increased. However, if those elements were plentifully supplied and if cropping were intensive, sulfur deficiencies might occur on many soils. Recently Freitas *et al.*, (1972) has reported responses from applying sulfur fertilizer on coffee in Sao Paulo state Brazil.

Sulfur deficiencies have been reported from other tropical areas including islands in the Caribbean (Hague and Walmsley, 1973) and Hawaii (Fox *et al.*, 1965). There is much less evidence for sulfur deficiency in areas of tropical rain forest. For example, Kang and Osiname (1976) grew corn in western Nigeria from the forest zone (Latitude 6° 55' N) across the area of derived savanna to the true savanna zone 9° 5' N). Responses to sulfate were obtained at all locations (except perhaps at the most humid locations), but the responses were more distinctly evident in the savanna zone. At one location in the savanna zone, 30 kg S/ha increased yield almost 100 percent over the control. The

Table 3. Status of sulfate-sulfur in a profile of Alagba soil--Ikenne, Nigeria[1/]

Depth Increment	Adsorbed Sulfate[2/]	Sulfate in Solution[3/]	Sulfate Adsorption Maximum	Sulfate Saturation Percentage: As Sampled	Sulfate Saturation Percentage: At 5 µg/ml in Solution
cm	µg S/g	µg/ml	µg/ml		
0-15	25	8.0	32	78	70
15-30	18	4.5	27	67	70
30-45	36	1.4	88	41	67
45-60	75	1.0	202	37	71
60-75	104	0.8	208	50	79
75-90	121	0.6	231	52	85
90-105	127	0.7	238	53	86
105-120	145	0.6	276	53	86
120-135	133	3.5	263	50	85

1/ Ife University Farm. Site had been in secondary forest regrowth about years prior to sampling.
2/ Four repeated extractions with $Ca(H_2PO_4)_2$.
3/ Sulfate-Sulfur in 0.01 M $CaCl_2$ at zero sulfate desorption.

Table 4. Elemental composition of 11 cultivars of cowpea grain produced at IITA, Ibadan, Nigeria and calculated quantities in harvested grain for two levels of production. (Data from Fox, Kang and Nangju, 1977)

Element	Concentration (%): Mean	Concentration (%): Range	Quantity in hypothetical harvests (kg/ha)[1/]: Typical	Quantity in hypothetical harvests (kg/ha)[1/]: Agronomically possible
N	3.97	3.64 - 4.36	8.9	59.7
P	0.47	0.44 - 0.54	1.1	7.4
K	1.63	1.50 - 1.80	3.7	24.4
Ca	0.10	0.10 0.11	0.2	1.5
Mg	0.23	0.21 - 0.23	0.5	3.4
S	0.25	0.21 - 0.28	0.6	3.8
Zn	0.0044	.0038 - .0051	0.01	0.07

1/ 224 kg/ha for typical yields; 1500 kg/ha believed to be agronomically feasible.

most humid location gave a very small, statistically non-significant, increase in yield. A reason for this result is evident from the data of Table 3. which was taken from the most humid (tropical rain forest) location. The sandy surface soil contained a modest amount of adsorbed sulfate with solubility of 8 µg S/ml. The subsoil, which contained more clay, was relatively rich in adsorbed sulfate but the solubility was low. It now seems probable that the better sulfur nutrition of crops in the forest zone is related to the capacity of the subsoils to retain sulfate. Although detailed data are lacking, savanna soils seem to be much poorer in absorbed sulfate.

From work done with several crops under a variety of conditions (Hasan *et al.*, 1970; Daigger and Fox, 1971; Fox *et al.*, 1976, 1977) it appears that the external sulfate-S requirements for several crops is about 5 µg/ml. From a standpoint of sulfate-S in solution, the Forest Zone soil seems to be adequately supplied.

In a recent review article (Summerfield *et al.*, 1974), cowpea *(Vigna unguiculata)* was placed among the grain legumes with immediate potential for alleviating human malnutrition. Cowpeas have relatively high protein content and are grown throughout the tropics and subtropics. The leading area of production is in the West African Savanna. Summerfield *et al.*, (1974) estimated the per season yield in Nigeria at about 224 kg/ha but it is now agronomically feasible to produce much higher yields. It is obvious from Table 4 however that agronomically feasible yields will require a much greater outlay of nutrients. We will examine what these numbers mean with respect to the sulfur supply.

Assuming typical yields of 224 kg/ha, a mean sulfur percentage of 0.25% (Table 4), and 50% partition of plant sulfur into the grain, a projected value of 1.12 kg S/ha in the cowpea crop is obtained. Mean annual sulfur in rainfall for northern Nigeria is about 1.14 kg/ha (Bromfield, 1974). These estimates suggest that cowpea production in the seasonally-dry savanna is already limited by nutritional constraints. Bromfield (1974) came to the same conclusion regarding groundnut production in northern Nigeria.

Concern about protein quality has led to renewed interest in the sulfur amino acid content of edible legumes including cowpea (Luse *et al.*, 1973). There is considerable interest in developing cowpea varieties with increased sulfur content. If this is accomplished and continuous cropping is instituted, the potential requirement for sulfur will probably exceed natural inputs into the agricultural system by an order of magnitude. Obviously the soils cannot sustain production with such a deficit. Even the forest zone soils must become sulfur deficient in time.

Whether or not it is possible to develop useful cowpea varieties which are rich in the sulfur amino acids remains to be seen. It certainly seems possible to exercise some control over protein quality by supplying sulfur to the plant. The data in Table 5 demonstrate that the concentrations of methionine and cyst(e)ine were increased approximately two-fold as sulfur nutrition was improved from severe deficiency to sufficiency for maximum yield, which for this variety was about 2 µg S/ml

Table 5. A comparison of the amino acid composition of cowpea meals obtained from the seeds of cultivar Sitao Pole grown with SO_4-S supplied at various concentrations in soil solutions. (Data from Evans, Boulter, Fox and Kang, 1976)

SO_4-S Concentration in Solution	Amino Acid Determined			
	Methionine		Cyst(e)ine	
µg/ml	(g/16g N)	(g/100g meal)	(g/16g N)	(g/100g meal)
None Added	0.64	0.181	0.43	0.117
0.2	0.95	0.305	0.32	0.104
0.6	1.11	0.333	0.50	0.150
1.8	1.15	0.320	0.80	0.222
5.0	1.24	0.349	0.92	0.258
15.0	1.12	0.294	0.94	0.263
45.0	1.30	0.362	0.98	0.274

in the soil solution. (Fox *et al.*, 1977).

We should not leave the subject of sulfur without saying a few words about burning. It has been estimated (McClung and Freitas, 1959) that about 75% of the sulfur contained in dry grass is volatilized during burning.

Volatization of anions during burning may have some advantage in that it produces basic plant ash and thus retards leaching of cations and development of soil acidity. However the practice is very wasteful of nitrogen and sulfur. Losses of sulfur for areas of shifting cultivation cannot be reckoned on the basis of crop removal since most of the savanna, in West Africa at least, is burned on an annual basis whether it is being cropped or not. Furthermore since burning is always done during the dry season there is almost no chance for recycling via rain or absorption of the sulfur oxides in moist soils.

Micronutrients

There seems to be a general consensus that micronutrient deficiencies do not, at this time, constitute major fertility problems of tropical soils. Isolated instances of deficiency have been recorded, however, and we suspect that what is now obvious is only the tip of the iceberg. We must not be caught unawares by assuming that highly weathered soils of the tropics can be brought to a high state of productivity by eliminating two or three nutritional problems.

Even at current levels of management one is impressed with the abundant signs of micronutrient stress in native gardens of West Africa. Probably in many cases these are associated with uneven distribution of ash (thus nonuniform pH) following burning--a problem which we will consider in more detail in a later section.

Frequently micronutrient problems are masked by more serious problems of another sort. One way to detect these multiple deficiency problems is to judge each experiment against the yield potential set by the prevailing conditions. Russell (1968) has pointed out that the yield potential in many parts of tropical Africa exceeds 6700 kg/ha for maize or sorghum, 2800 kg/ha for groundnuts and 2200 kg/ha for seed cotton. Experimenters should pay more attention to the reasons why yields on their experimental plots do not attain these levels. Low crop yields and lack of response to major nutrients often mean that there are other nutrient deficiencies that must be rectified before further experimentation is worthwhile.

Obviously we cannot deal here with all of the micronutrients that have been reported as deficient in the tropics. Only one will be discussed.

Molybdenum. A discussion of molybdenum nutrition is especially appropriate for this occassion since nitrogen fixation depends on an adequate molybdenum supply.

Molybdenum deficiencies have been encountered in soils developed in highly weathered parent materials both in and out of the tropics. In West Africa deficiencies have been reported in groundnuts and in East Africa on some high altitude pastures. In Hawaii molybdenum deficiencies have been encountered in alfalfa growing on the Wahiawa soil (Young and Takahashi, 1953) and for soybean growing on the same soil (R.L. Fox, unpublished data). This is not a surprising result since the Wahiawa soil is acid, consists almost entirely of clay sized particles, is high in iron oxides and is noted for its high manganese status. It also absorbs much phosphate. Barrow (1970) and Reisennauer *et al.*, (1962) have reported on molybdenum sorption by clays. In many respects molybdate behaves like phosphate except that phosphate adsorption is less pH sensitive. Younge and Takahashi (1953) reported molybdenum requirements an order of magnitude higher than were typical for the sandy soils of Australia.

Molybdenum is required in such small amounts that seed treatment with molybdate may make a significant contribution toward crop requirements. However, in acid soils which are highly reactive to anions such as phosphate, sulfate and molybdate, seed treatment probably will be relatively ineffective. In such cases high molybdenum seed may supply Mo relatively more effectively.

Some results of an experiment by Gurley and Giddens (1969) which illustrates several points concerning the molybdenum nutrition of soybeans is presented in Table 6. Note that soybeans growing on two soils, a loam and sandy loam, developed from highly weathered parent materials responded to molybdenum additions but that soybeans growing on the Norfolk Sand did not.

Note further that soybean seeds which carried 19 μg Mo/g seed (apparently most soybean seeds in the USA contain <2.5 μg/g of Mo (Harris *et al.*, 1965) did not fully meet the needs of the crop growing on molybdenum deficient soil. There is little carry-over of molybdenum

Table 6. Influence of seed composition and foliar applied molybdenum on the yield of soybean grain grown on soils representing a range of adsorption capacities. (Data from Gurley and Giddens, 1969).

Treatment		Soil		
Mo Content of Planted Seed	Mo Applied Foliar Spray	Norfolk Loamy Sand	Hiwassee Loam	Cecil Sandy Loam
µg/g	g/ha	grain yield kg/ha		
0.3	--	2,433	1,505	632
0.5	28	2,419	2,009	954
19.0	--	2,392	2,332	1,035
48.4	--	2,513	2,755	1,304

Table 7. Influence of nitrogen fertilization on the yield and protein content of soybeans grown in the Humid Tropics IITA, Ibadan, Nigeria (Luse *et al.*, 1975)

N added (kg/ha)	Seed yield (kg/ha)		Protein yield (kg/ha)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
0	1110	1140	340	410
30	1140	1700	330	580
60	1640	1800	500	660
120	1760	2010	640	760

Table 8. Response of soybean to molybdenum applied as a seed treatment before planting in relation to the molybdenum content of grain grown from untreated seed. (Data recalculated and rearranged from data of Lavy and Barber, 1963).

Mo content of untreated seed (µg/g		Number of Trials	Mean yield of grain (No Mo) (kg/ha)	Yield increase from added Mo (%)
Mean	Range			
0.7	0.6 - 0.9	3	2310	11.3
1.2	1.1 - 1.2	4	2510	3.7
1.6	1.5 - 1.6	2	2110	3.5
2.8	1.7 - 3.5	3	2450	0.6
6.8	5.4 - 8.2	2	2460	-1.1

into seed of the second generation (Gurley and Giddens, 1969).

These data are interesting in view of nitrogen deficiency symptoms frequently encountered during early growth of soybean at IITA in Nigeria and the responsive nature of soybean to nitrogen even when they have been inoculated (Kang 1975). The problem is no doubt intensified if varieties are used which flower prematurely under low light intensity, short days conditions prevailing at low latitudes. Early flowering, creates a large sink for photosynthate thus depriving the N_2-fixing bacteria of the energy source needed for effective N_2-fixation. One example of the influence of N-fertilizer on soybeans grown at IITA Ibadan, Nigeria is presented in Table 7.

One of us (R.L. Fox) examined the molybdenum contents of 26 soybean seed samples produced in uniformly fertilized plots at Ibadan. The molybdenum values were very low--in all cases <0.2 μg/g. Two methods of ashing, dry and wet combustion, were used with similar results. These data have not been confirmed; but even if the values are higher by a factor of three or four, a deficiency of considerable importance for legume growth is indicated. Data of Lavy and Barber (1963), reorganized and presented in Table 8, suggest that soybeans containing <2 μg/g of Mo may have come from deficient plants.

The evidence for molybdenum deficiency in the tropics is, as yet, not extensive but there are enough indications to suggest that this might be a profitable area for research. The work of Lavy and Barber (1963), Harris *et al.*,(1965) and Gurley and Giddens (1969) suggests that seed analyses would provide an excellent technique for surveying the subject. Unfortunately at this time we do not know what the critical levels for seeds, other than soybeans, may be.

We have little information on molybdenum adsorption by soils in the tropics. Such information is needed before a reasonable approach can be made to the practical aspects of fertilization.

SOME FERTILITY PROBLEMS INVOLVED IN EXPANDING AGRICULTURAL DEVELOPMENT IN THE TROPICS

Traditional systems of shifting cultivation were developed by cultivators through centuries of trial and error to a stage where some sort of equilibrium with natural forces was attained. Considering the resources and technology available to the primitive cultivators, traditional farming systems were often efficient systems (Kang and Okigbo, 1975). The main criticisms against traditional shifting agriculture are low yields and the small proportion of land in current production. Shortening the fallow period of eliminating it altogether is an obvious way to increase production <u>if production can be maintained</u>. There are several problems. These can be summarized as (1) Deterioration of the chemical and physical properties important to soil fertility, (2) Build-up of weeds, insects and diseases.

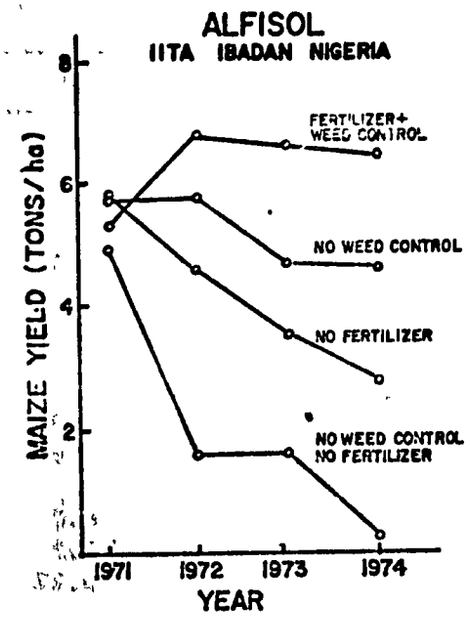

Figure 5. Production of maize at Ibadan, Nigeria in relation to fertilizer applications and weed control. The fertilizers used and rates of application were designed to provide what was deemed to be optimum yields of maize.

Soil Fertility Problems Associated With Continuous Cultivation

It is almost universal experience that highly weathered and leached soils rapidly lose their native fertility after they are brought into production. Doubt is sometimes expressed about whether or not production can be maintained during continuous cropping in the tropics even with fertilizers. Russell (1968) has reviewed some of the evidence on this subject from Africa. Unfortunately there are not many good practical experimental examples. Some experiments involve unrealistic amounts of animal manure or mulches. In other cases soils have been acidified to their detriment by ammonium sulfate. However, there are numerous commercial examples which show that, with adequate fertilization and other management practices, sustained high production can be had from highly weathered soils. Sugar cane and pineapple production in Hawaii is world renowned in this respect.

The data presented in Fig. 5 demonstrate the magnitude of problems which would confront farmers in much of the humid tropics if they should attempt to produce a succession of grain crops with no nutrient additions or weed control. On the other hand there is evidence that fertility can be maintained by judicious soil management.

The problems which arise as a result of continuous cropping are complex of course and many problems itensify because of year-found equable temperature. Very little is known about which factors most limit crop production on degraded land. Soil physical problems may share equally with nutritional problems. Prolonged cropping has been

Table 9. Some Chemical and Physical Properties of soils in relation to distance from oil palm trees, Egbeda Soil, IITA, Ibadan Nigeria (Kang, 1977)

Soil Property	Distance from oil palm tree	
	0.5 meter	3 meters
Particle size (%)		
Sand	68.0	72.0
Silt	18.0	14.0
Clay	14.0	14.0
Bulk density (g/cc)	0.67	1.16
Moisture Content 1/3 Atmos.(%)	16.5	10.5
pH (water)	6.2	6.5
Organic C %	2.43	1.33
Cation Exchange Capacity (me/100 g)	13.2	5.9
Exchangeable Cations (me/100g)		
Ca	8.7	4.0
Mg	3.3	1.3
K	0.49	0.27
Na	0.10	0.07
Mn	0.29	0.13
Extractable P (Bray No.1) (µg/g)	2.5	1.8

Table 10. Influence of accumulated ash on burned sites on the chemical properties of the soil. Alluvial Terrace Soil, Niger River Delta Nigeria (unpublished data of B.T. Kang, IITA)

	Sites where burning was intense	Adjacent sites
pH (water	5.7	4.5
Organic C (%)	1.7	1.6
Exchangeable Cations (me/100g)		
Ca	2.1	1.1
Mg	0.7	0.3
K	0.25	0.09
Extractable P (Bray #1) ppm	161.0	133.0

associated with increasing soil bulk density and soil crusting.

Many of the fertility problems, both chemical and physical, are linked to soil organic matter decline. While the availability of nitrogen and sulfur may temporarily improve as the C:N and C:S ratio become more narrow immediately after fallow, in most soils of the

savanna this is not sufficient to meet the demands of intensive crop production. Phosphorus deficiencies, which may not be severe immediately after burning the accumulated vegetation of the fallow years, become more intense with time (Jones and Wild, 1975).

The rate at which total carbon is mineralized when fallow land has been recultivated has been variously estimated at 4.5% to >10% per year.

New deficiencies, especially potassium, may appear as time progresses especially in deeply weathered soils.

Soil Microvariability in Relation to Soil Fertility

A prominent feature of many tropical soils is microvariability associated with biological activity of various kinds,(Kang and Moorman, 1977). For example the average density of termite mounds at the IITA, Ibadan, Nigeria, facility was 32 per ha. Termite mounds are just one of several sources of microvariability.

By experience, native cultivators know the differences in productivity of the various soils within their holdings and will select crops, cropping sequences and management practices that will adjust best to the soils. They also know about how long the cropping period should last, or at least when to rest the soil.

This kind of detailed attention is not feasible when extensive agriculture is practiced. During early stages of development of agricultural lands, microvariability will be especially great. The judicious use of fertilizers can help bring land into uniform production but the effect may not be eliminated in this way because much of the problem is related to soil physical conditions.

Examples of how oil palm trees, termite mounds and burning can influence soil chemical and physical properties are presented in Tables 9 to 11. The data in Table 12 illustrates the adverse effects of termites and the beneficial effect of unfertilized oil palm trees grown in traditional farmer's plots on cowpea grain production at one location in Nigeria.

From whatever source, microvariability can lead to a wide range of production within small areas. Some results of one study at IITA, Ibadan, Nigeria are outlined in Table 13. Soybean leaf samples, nodule samples, and associated soil samples were taken from 26 points in uniformly fertilized soybean plots (about 0.2 ha) belonging to Dr. Ratan Lal. The soybean plants ranged from yellow-green, stunted plants to very dark green, vigorous plants. Dark green color was associated with good nodulation, high nitrogen fixation, high leaf nitrogen percentage and eventually, good yields of high protein grain. Much of the variability apparently was associated with leaf manganese, which in excess of about 325 µg/g leaf tissue, was a constraint on N fixation (Fig. 6). Leaf manganese, in turn was associated with soil pH; soil pH (0.01 M $CaCl_2$) of below about 5.8 was associated with plant manganese >325 µg/g (Fig. 7). The subject of lime response in a tropical environment is covered in detail by Abruna *et al.*, (1975).

Table 11. Characteristics of soil derived from termite mounds compared with soil between mounds Egbeda Soil, Ibadan Nigeria (Unpublished data of B.T. Kang, IITA)

Soil Property	Termite Mound	Between Mounds
Particule Size (%)		
Sand	54.0	59.0
Silt	15.0	19.0
Clay	31.0	22.0
Organic Carbon (%)	0.76	1.56
Exchangeable Cations (me/100g)		
K	0.43	0.48
Ca	2.61	3.74

Table 12. Yield of cowpea grain in relation to factors contributing to soil microvariability Egbeda Soil, Ibadan Nigeria (Unpublished data of B.T. Kang, IITA)

Soil Condition	Yield (g/plant) 1/
Termite mound site	4.5
Control site	9.7
Palm Tree site	15.5

1/ All values statistically different (5% significance) from each other.

The second most important factor associated with soybean leaf color and grain yield was leaf potassium percentage. The relationship for color v leaf K is presented in Fig. 8. The solid circles represent levels of Mn that are nontoxic or only slightly so.

The point we wish to make is that two factors, manganese toxicity due to soil acidity, and potassium deficiency, neither of which have been ranked as major soil fertility problems in the tropics appear to be responsible for much of the variability we encountered in this case. We doubt if this is just an isolated example. Obviously much more needs to be done before we can speak with certainty about major fertility problems of tropical soils.

SUMMARY AND CONCLUSIONS

It is probably safe to say that there is nothing unique about the type of soil fertility problems encountered in the tropics. What is

Table 13. Soil and crop data collected at 26 points in plots of uniformly fertilized soybeans. Minimum tillage plots. IITA Ibadan Nigeria. Plots sampled Oct. 1974 at first pod set. (Field materials supplied by Dr. Ratan Lal)

Soil or Plant measurement	Range
Soil pH (water)	5.1 - 7.0
(0.01 $CaCl_2$)	4.5 - 6.8
Exchangeable Cations (me/100g)	
Ca	1.7 - 7.2
K	.15 - .34
Mg	.13 - .83
Al	.04 - .20
Nodules	
Number per plant	3.6 - 56.2
g/plant (dry wt.)	.05 - .33
Nitrogen fixation	
µM/plant	2.4 - 66.4
µM/ g nodule	28 - 229
Plant nitrogen	
Upper trifoliate leaf (%)	2.46 - 4.82
Plant top (mg/plant)	25 - 174
Plant Manganese (µg/g)	243 - 538
Grain Yield (kg/ha)	900 - 2600

unique is the magnitude of soil fertility problems in relation to quantities of resources which are being committed to alleviate those problems. Some widely-held attitudes about soil fertility in the tropics need to be changed before substantial progress can be made. Three of the most obvious are:

1. The traditional farmer cannot afford cash inputs
2. The first increment of input gives the most economic yield increase.
3. What is really needed is a new crop or variety which can thrive regardless of soil infertility.

Subsistence agriculture in much of the tropical zone is operating against a soil fertility constraint. Without substantial fertility inputs there is little likelihood for sustained, increased production.

Soil fertility problems even in the humid tropics are those common to highly weathered and leached soils elsewhere in the world.

For legumes, phosphorus deficiency is the single most important soil fertility problem in the tropics. In some soils intensity of phosphorus nutrition is low and capacity for phosphate adsorption is high. Such soils will require massive phosphate applications before they will be highly productive for most crops. Such soils may continue to sustain low production for many years. Many soils of the tropics,

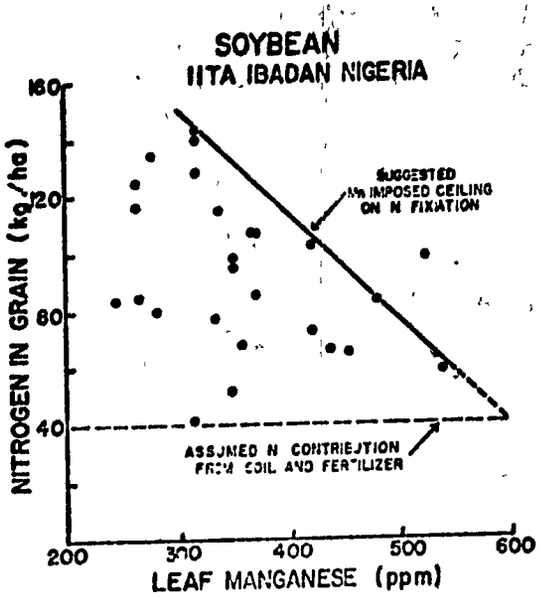

Figure 6. Nitrogen in soybean grain in relation to manganese in the top, fully-developed trifoliate leaf.

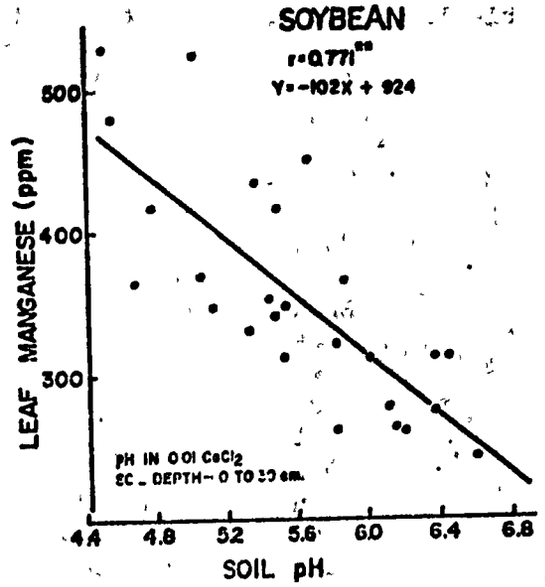

Figure 7. Manganese concentration in soybean leaves in relation to soil Ph.

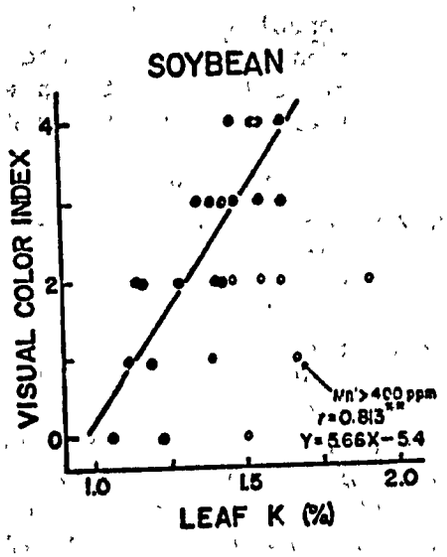

Figure 8. Color of soybean plants in relation to potassium in the top, fully-developed trifoliate leaf.

including much of west Africa are sandy. These soils have relatively low capacity to adsorb phosphorus. Modest applications of phosphate fertilizers should suffice for most crops but, unless phosphate is recycled in the soil system, frequent applications of phosphate will be required.

Long term field experimentation is needed to establish the external P requirement of important crops and to plot the pattern of phosphate fertilizer requirements as a function of time. Few long term trials have been conducted, but the information available indicates much better residual effects from phosphate than had previously been expected.

If phosphorus is deficient the most useful approach to solving the problem is the direct approach: apply phosphate. Manipulation of soil pH, modifying ionic strength of soil solution, displacing phosphorus with inorganic or organic anions, solubilizing and utilizing native phosphorus with especially adapted roots and bacteria, mychorrizal association with roots, and specialized placement of phosphate fertilizers - all are useful techniques in their place but they are secondary to establishing and maintaining a good base level of phosphate in the soil.

The use of supplemental foliar applied phosphate has given some promising results and should be investigated further.

If the phosphate and nitrogen problems are solved, sulfur deficiencies will become general in the tropics unless supplementary sulfate-containing fertilizers are used. Some soils of the humid tropics contain much adsorbed sulfate in the subsoil. The solubility of this sulfate is low and it is replenished slowly.

Deficiencies of potassium, calcium and magnesium are "waiting in the wings."

Micronutrient problems are not widely recognized in the tropics probably because other constraints are more limiting. No doubt micronutrient problems will intensify as crop production is increased.

Toxicities of aluminum and manganese are serious problems in many areas. Problems will increase as nitrogen fertilization is more widely practiced.

There are many soil management problems in the tropics. Physical and chemical properties deteriorate rapidly if cultivation continues without benefit of fertilizers and good crop residue management. There is evidence that continuous cultivation can be practical but research to identify methods which ensure good long term results is urgently needed for the various soil types.

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SOIL ACIDITY AND RELATED FACTORS

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INTRODUCTION

Much of the detailed knowledge of soil acidity effects on legumes pertains to certain temperate agricultural species. Twenty years ago, Dr. D.O. Norris refuted any tendency there might have been to extrapolate this limited experience too freely. He argued that tropical legumes and their rhizobia should tolerate soil acidity considerably better than their temperate counterparts (Norris 1956, 1959). The usefulness of this generalization is now compromised by accumulated exceptions (Andrew, 1976, Foster, 1970, Lee and Wilson, 1971, Loneragan, 1973, Munns, 1976, Munns and Fox, 1976, Norris, 1965, 1967, 1971, 1972). But the main message remains clear: one does not confidently deduce the behavior of any legume, tropical or temperate, from experience with another. Therefore this review will include information from legumes of all persuasions, as being likely to have equal relevance (or irrelevance) to the understanding of any particular tropical species.

In soils there tends to be some correlation between acidity, calcium deficiency, manganese toxicity, and aluminum toxicity. All four are corrected by liming. Their biological effects tend to be interactive. These are the reasons for dealing with these four "soil-acidity factors" together. However, although the factors do correlate, the correlations are not close. Aluminum availability does relate fairly closely to soil pH in mineral soils (although acid organic soils may be quite unlikely to support toxic levels of free aluminum). However, in numerous acid soils neither calcium deficiency nor manganese toxicity is much of a problem; and severe calcium deficiencies and manganese toxicities do occur in soils that are not very acid or high in free aluminum (Loneragan, 1973, Pearson, 1975, Pearson and Adams, 1967). This degree of independent variation in the four factors has consequences. For one, it means that soil pH is not the most reliable indicator of trouble. For another, it means that it is useful to attempt to identify which of the factors are most strongly affecting a particular legume in a particular area.

Any soil amendment can alter each of the soil-acidity factors, and many other soil properties as well (Barrow, 1965, Pearson, 1975, Pearson and Adams, 1967). There are important documented cases of lime-induced changes in the availability of aluminum, manganese, potassium, magnesium,

zinc, copper, boron, iron, phosphate, sulfate, molybdate, and nitrogen. Most of these changes can also be expected when the soil pH is amended with noncalcic bases (e.g. sodium, magnesium, or potassium carbonates), except that these are more likely to lower calcium availability than to increase it. The addition of a neutral calcium salt likewise does more things than simply raise the concentration of soluble calcium. It may significantly lower the soil pH, and increase the availability of aluminum, manganese, and other cations, unless the displaced cations and acidity are removed by leaching. The effect of salt concentration on pH and aluminum solubility helps explain observations that drought aggravates effects of soil acidity on legume growth and nodulation (Baker, *et al.*, 1968). It also means that soil pH data have little meaning unless the soil: water ratio or the salt concentration of the suspending solution is specified. (Measurements made in dilute aqueous suspensions tend to overestimate by 0.5 to 1.0 unit the pH of a saturation paste, of a displaced soil solution, or of a suspension in 0.01M $CaCl_2$ (White, 1969).

None of this helps towards easy, confident interpretation of soil experiments. Experimentation with simplified media, e.g. solution culture, becomes a useful supplementary research tool. In solution cultures, effects of pH, calcium, manganese, and aluminum can be separated to a degree from each other and from other effects. Also, agitated or flowing cultures reduce concentration gradients due to diffusional limitations which operate in solid media near the root. However, the simplications possible are limited, rather obviously by such things as the limited solubility of calcium and aluminum phosphates and aluminum hydroxide, and the simplicity may be illusory. In particular, it can be difficult to maintain the supposed constancy of composition of the medium against changes caused by plant and microbe. Few simplified media are as well buffered against compositional change as soils are. To counteract the changes requires frequent solution replacement, frequent adjustment, or the use of flowing cultures (Asher and Loneragan, 1967, Epstein, 1972, Loneragan and Snowball, 1969).

Any nutritional disorder may, in principle, differently affect each separate phase of the legume/rhizobium symbiosis (e.g.(I) rhizobial survival in the soil and growth in the rhizosphere (II) infection and nodule establishment (III) nodule function and (IV) growth of the host plant). To distinguish which phase is primarily affected may be difficult, either because of lack of data, or because the phases have closely similar tolerances, or because the effects are so closely linked that their distinction becomes artificial. But where distinction is possible, it is useful to attempt it: it bears on the choice of corrective measure, especially when the choices include adaptation of either the host or rhizobial genotype, rather than just correction of the soil.

Growth of the host is tied closely to other phases of the symbiosis. Thus a nutritional disorder may limit fixation "indirectly", by impairing growth of the host plant with a consequent decrease in nodulation and nitrogen fixation. This case can be operationally defined by lack of response to combined nitrogen. It contrasts with "direct" inhibition of nodulation or nitrogen fixation, where the plant's

nitrogen shortage diminishes its growth, and response to combined nitrogen is positive. Amongst the soil-acidity factors, available information implies that calcium and pH are most likely to affect nitrogen fixation directly, whereas aluminum and manganese are likely to affect it indirectly, by inhibiting host plant growth.

THE HOST PLANT

Legumes have had a reputation for intolerance of soil acidity and high calcium demand, but the family as a whole does not deserve it. It is true that certain legumes tolerate soil acidity less than certain other plants; and that nodulation and nitrogen fixation may add special requirements. But even when dependent on nitrogen fixation, some legume species grow well in media that are extremely acid, low in calcium, or high in manganese (Andrew, 1976, Dobereiner and Aronovich, 1966, Munns, 1976, Munns and Fox, 1976, Pearson, 1975, Souto and Dobereiner, 1968, 1969). These species include *Vigna unguiculata, Lotononis bainesii, Macroptilium lathyroides,* certain *Lupinus* and *Pueraria* species, and certain subspecies of *Trifolium africanum*. When not dependent on nitrogen fixation, legumes probably have the same range of tolerances as would be expected in other large diverse groups of plants.

When grown with adequate combined nitrogen, most of the legumes studied do not become calcium deficient until their internal (shoot or leaf) calcium concentration is well below 0.5% (Andrew, 1976, Loneragan *et al.*, 1968). The much higher levels often found, 2% or above, evidently represent luxury consumption responses to the high levels of soluble calcium in many acid soils and most nearly neutral soils. Critical external calcium concentrations in controlled solution or sand culture experiments well below 1 me/liter have been demonstrated for several legumes (Andrew, 1976, Loneragan *et al.*,1968, 1969). Species with low internal and external requirements for calcium include certain *Lupinus* and *Ornithopus* species, (Asher and Loneragan, 1967, Loneragan *et al.*,1968, 1969), *Lotononis bainesii, Macroptilium lathyroides, Stylosanthes* species, *Vigna unguiculata, Arachis hypogea* (for growth, not seed set), *Desmodium uncinatum,* and even such reputedly calcium-demanding species as *Medicago sativa, Medicago truncatula,* and *Trifolium repens*.

Normal tolerance of low pH has been demonstrated in legumes given ample combined nitrogen and in some species ample calcium in solution. Under these circumstances, even *Medicago sativa, Glycine wightii,* and some *Trifolium* species can grow almost normally at pH4 (Andrew, 1970, Helyar and Anderson, 1971, Munns, 1965), although lowering the calcium concentration much below 1 me/liter reduces their acid tolerance. Other species, such as *Lotononis bainesii, Desmodium uncinatum, Macroptilium lathyroides,* and *Stylosanthes* species have shown no positive response to increase of pH above 4 (Andrew, 1976). In *Pisum sativum* and *Medicago sativa,* pH below 5 which inhibits nodulation does not prevent normal rates of root growth and root hair production (Lie, 1969, 1974, Munns, 1968). So far, solution culture experiments in general indicate

that growth of a legume fed with combined nitrogen is less sensitive to acidity or calcium deficiency than is nodulation or nodule function in the same legume (Andrew, 1976, Loneragan, 1973, Munns, 1965, 1967, Vincent, 1965).

Some of the above experiments show that calcium and pH interact on the growth of legume plants, just as they do on growth of non-legumes (Epstein, 1972). Further, the effects of extremely low pH resemble those of calcium deficiency in that the roots become stubby, stunted and discolored. In addition, severe calcium deficiency may cause petiole collapse (Andrew and Norris, 1961, Epstein, 1972).

Manganese toxicity tolerances vary considerably between and within legume species (Andrew and Hegarty, 1969, Loneragan, 1973, Munns, 1976 Robson and Loneragan, 1970). Some *Medicago* species are sensitive to as low as 0.3 µM manganese ion in solution cultures. Critical levels of leaf or shoot manganese for some different species are listed in Table 1. Raising the concentration of calcium mitigates the adverse effect of manganese (Loneragan, 1973, Robson and Loneragan, 1970, Truong *et al.*,1971, Vose and Jones 1963). Raising the pH, on the other hand, increases the uptake of manganese and the severity of toxicity in solution cultures with a fixed manganese concentration (Robson and Loneragan, 1970, Truong *et al.*,1971). This tendency opposes the tendency for manganese solubility in acid soils to decrease when pH is raised, and may explain why liming need cause little decrease in plant manganese content (see Figure 1). Increasing phosphate supply can aggravate manganese toxicity in solution cultures and soils (Munns, 1976, Truong *et al.*, 1971). The common symptom of manganese toxicity is chlorosis between veins and withering of leaf tips and margins, whether or not combined nitrogen is supplied.

Manganese toxicity has not yet been clearly shown to directly inhibit nitrogen fixation (Loneragan, 1973, Munns, 1976). Tolerance limits for host plant growth may be close to those for optimal nodulation and nodule function. As an example, lime pelleting of *Centrosema pubescens* planted in a high-manganese Brazilian soil improved nodulation; but to reduce manganese uptake, increase growth, or increase nitrogen fixation, required liming of the whole root zone (Dobereiner and Aronovich, 1966).

Aluminum toxicity becomes important to plants in severely acid soils. Growth of sensitive legumes such as *Medicago sativa* and M. *scutellata* is depressed significantly by 20 µM aluminum in solution, likely to occur in soil at pH 5; and is stopped by 70-80 µM aluminum (Andrew *et al.*, 1973, Munns, 1965). Very insensitive species, unaffected by 75 µM aluminum, include *Desmodium uncinatum, Lotononis bainesii, Macroptilium lathyroides*, and *Trifolium rueppellianum* (Andrew *et al.*,1973). Species of intermediate sensitivity include *Trifolium subterraneum* (Munns, 1965), T. *semipilosum*, T. *repens*, and *Glycine wightii* (Andrew *et al.*,1973).

Aluminum toxicity can reduce the uptake and translocation of calcium and phosphate (Andrew *et al.*,1973, Clarkson, 1965, Epstein, 1972, Munns, 1965) and its severity can be diminished by raising the concentrations of these nutrients in the substrate (Munns, 1965). In addition, heavy phosphate fertilization of soil precipitates free

aluminum, making phosphate an effective though unneccessarily expensive remedy for aluminum toxicity (Munns, 1965). The symptoms of aluminum toxicity are stunting and stubbiness of the roots, and shoot symptoms resembling phosphate deficiency but not necessarily connected with low phosphate content (Andrew *et al.*,1973, Munns 1965). All these effects in legumes are consistent with observations on non-legumes (Epstein, 1972, Pearson and Adams, 1967).

Aluminum toxicity in symbiotically-grown legumes has received virtually no study. It would seem unlikely to have much importance in the soil-pH range in which many legumes grow well symbiotically, except in the case where effective nodulation was attained by lime pelleting or localized application of lime to only a part of the root system, leaving the rest of the soil uncorrected. However, aluminum toxicity may prove to be important in some legumes capable of functioning well symbiotically at low pH.

EFFECTS OF THE PLANT ON THE MICROENVIRONMENT IN THE RHIZOSPHERE

Changes in inorganic composition induced by the plant might drastically alter behavior of rhizobia in the rhizosphere as compared to their behavior in the soil at large; and the chemical environment of the rhizosphere is that which directly impinges on root and nodule. However, this whole subject remains an area of quantitative ignorance.

The plant's effects probably for outweigh any effect rhizobia might exert. The plant's effects would be amplified by limitations on diffusion in the soil or in sand, gravel, agar and other solid media. They are probably minimized in liquid media, though even here the existence of a stationary boundary layer on the root should cause some gradation of properties, however microscopic; and this gradation would be magnified by the presence of "mucigel" observed on roots of various legumes grown in solutions (Dart, 1971, Dart and Mercer, 1964, Lie, 1969, 1974, Munns, 1968).

The concentration gradient of any ion species in the rhizosphere depends on its rate of uptake or release by the root, on the inward flux of water to meet transpiration, and on the ion's diffusivity in the medium and its concentration in the bulk soil solution outside. Sorption and desorption by solid surfaces will tend to buffer the ion's concentration and also decrease its apparent diffusivity. Ions which are strongly sorbed, rapidly taken up, or low in concentration in the soil solution tend to become depleted near the root: ions which are weakly sorbed, slowly taken up, or plentiful in solution tend to accumulate near the root if there is vigorous transpiration. (Barber and Ozanne, 1970, Riley and Barber, 1969, Wilkinson *et al.*,1968).

What data exist include radiographic demonstrations of calcium accumulation at the roots of *Trifolium subterraneum* (Barber and Ozanne, 1970), and analytical evidence of a rise in pH, bicarbonate, and calcium at the roots of *Glycine max* (Riley and Barber, 1969). These were presumably observed in soils moderately high in pH and calcium. Where calcium is deficient (e.g. at concentrations much below 1 me/liter) its

Table 1. Critical Concentrations of Manganese in Shoot Tissue. (ppm) (level at which 10% yield reduction occurs)

Centrosema pubescens	1600	*Macroptilium lathyroides*	850
Desmodium uncinatum	1260	*Medicago sativa*	460
Glycine max	200-1000	*Medicago truncatula*	660
Glycine wightii	600	*Phaseolus vulgaris*	1000
Lespedeza sp	500	*Stylosanthes humilis*	1150
Leucaena latisiliqua	550	*Trifolium fragiferum*	500
Lotononis bainesii	1300	*Trifolium repens*	650
Macroptilium atropurpureum	800		

Sources: Andrew and Hegarty, 1969, Aust. Res. 20,687
Martin and Matocha, 1972, *In* "Soil Testing and Plant Analysis" Amer. Soc. Agron.
Chapman, 1966, "Diagnostic Criteria for Soils and Crops." Univ. Calif. Agr. Exp. Sta.

Note: Critical levels vary depending on plant part analyzed and stage of growth.

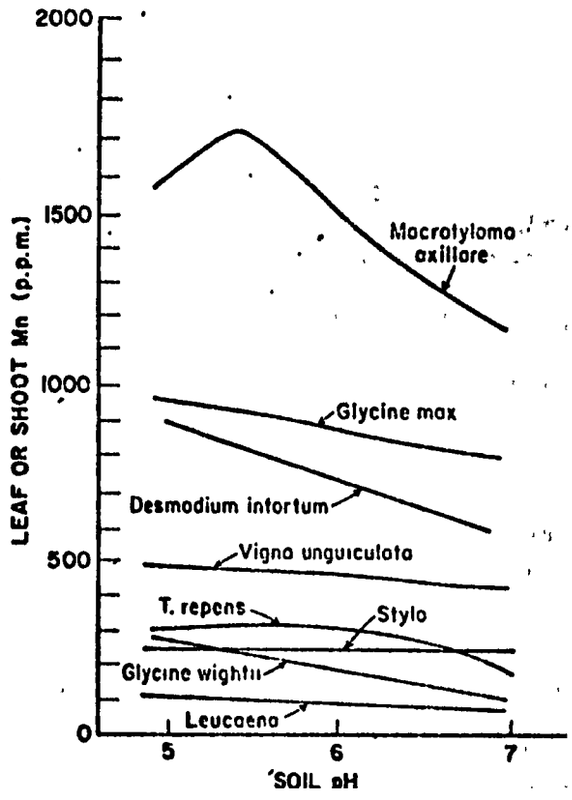


Figure 1. Effects of liming on manganese contents of legumes grown on Wahiawa silty clay, Poamoho, Oahu, Hawaii. (From data of Okazaki, Fox, Minna, unpublished).

concentration should be even further lowered at the root surface of a "normal" legume transpiring at normal rates (Munns and Fox, 1977). Similar argument would indicate that at toxic levels aluminum, and perhaps even manganese, should tend to accumulate to higher concentrations near the root than in the bulk soil. An exceptionally immobile nutrient needed in large amount, like phosphate, rapidly drops to almost vanishing concentration in the root hair cylinder (Helyar and Munns, 1975). Depletion of phosphate, apart from its direct consequence to rhizobial phosphate nutrition, might alter the availability of aluminum and the need for calcium.

Differences in pH between rhizosphere and bulk soil can only be guessed. The efflux of hydrogen ions from nitrogen-fixing legumes can eventually be large enough to shift the whole soil pH downward, because there is little of the nitrate uptake which in ordinary plants balances the uptake of nutrient cations (Nyatsanga and Pierce, 1973). However, the released hydrogen ions may cause little pH-change in the rhizosphere because of their ready outward diffusion and the soil's pH-buffering. In Figure 2, nodulation of *Medicago sativa* in a collection of acid soils is compared with nodulation of the same species in solution cultures. (Calcium was fairly high in all cases.) Perhaps we can regard the nodulating alfalfa root here as a biological pH-meter, calibrated in

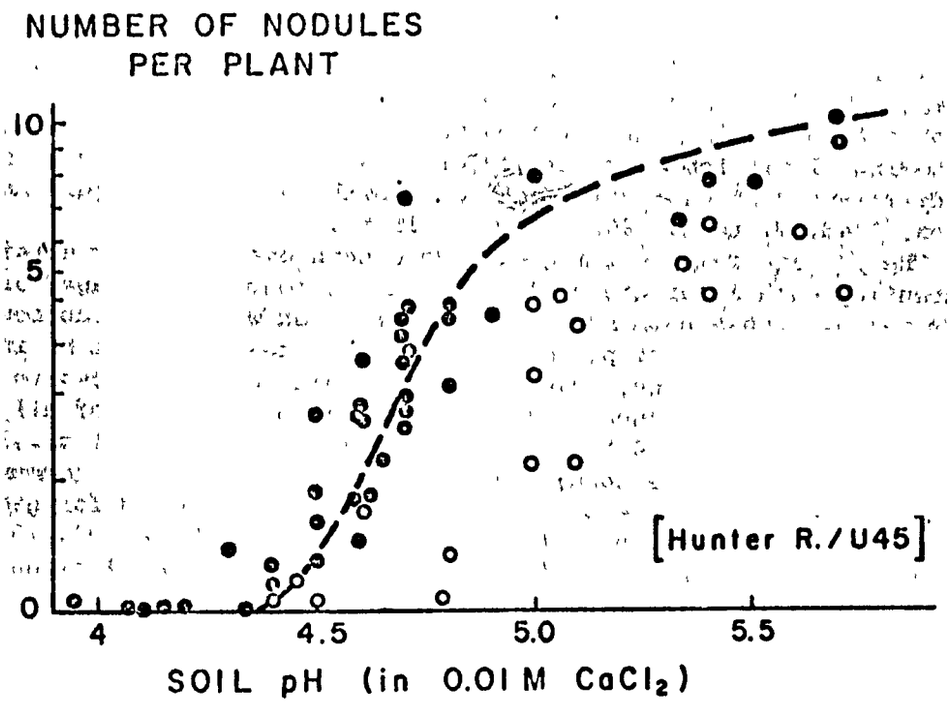


Figure 2. Nodulation of *Medicago sativa* in relation to substrate pH. Curves are drawn from results of trials in solution cultures with 3-6 mM calcium (Munns, 1970). Points represent data from a greenhouse trial with 29 soils from southern New South Wales, each with and without calcium carbonate addition (Munns, unpublished). Open circles represent soils in which nitrate was high enough to inhibit nodulation.

solution culture, and inserted into its own rhizosphere at the critical time of nodule initiation. If so, it tells us that in these soils the rhizosphere had the same pH as that measured conventionally in the bulk soil. Alternatively, it may be telling us it is a miscalibrated pH-meter: that there is a rhizosphere effect in the solution cultures (mucigel?) just as large as in the soils. The question may be resolved by someone rash enough to use ultramicro glass electrodes with confidence.

RHIZOBIA

Nodulation difficulties in acid soils can be caused or aggravated by poor rhizobial survival and colonization. Moderate inhibition of nodulation by acidity in soil and solution culture can sometimes be overcome partly by increasing the size of the inoculum (Anderson, 1970, Jensen, 1969, Robson and Loneragan, 1970, Spencer, 1950), or by waiting long enough for a large population of competent rhizobial types to build up (Anderson and Moye, 1952). These interactions between acidity and inoculum size may merely mean that fewer bacteria are needed for infection if pH is favorable. But the alternative explanation, that the effect of acidity on nodulation includes inhibition of rhizobial growth, is consistent with data on the distribution of rhizobia in soils, at least for *R. trifolii* and *R. Meliloti*. *Rhizobium meliloti*, for instance, is rarely abundant in soils with saturation paste pH much below 5, and *Rhizobium trifolii* in soils with pH below 4.5 (Jensen, 1969, Peterson and Gooding, 1941, Robson *et al.*,1970). These data are in turn consistent with differences in pH requirement for growth in agar or liquid media (Jensen, 1943, Vincent, 1962, Vincent, 1965).

The pH requirements for growth have been evaluated for strains representing each major group of rhizobia, in conventional agar or liquid media. These data have to be interpreted with some caution because of uncertainty of pH-control and the likelihood that pH interacts with other factors (Munns, 1976). Granted this perhaps excessive caution, strain differences seem to be large: some strains of all groups cannot grow at pH below 4.5 (Graham and Parker, 1964, Ham *et al.*,1971, Jensen, 1942, 1963, Loneragan, 1973, J.M. Vincent, personal communication). Within *R. japonicum*, there are serogroups whose minimum pH for growth varies as much as three units (Damirgi *et al.*,1967, Ham *et al.*,1971). The only confident generalization possible is that *R. meliloti* has few strains capable of tolerating pH 4.5 or lower. The pH requirements for growth of most useful strains may be close to those for nodulation of the respective host. (This does not mean that low pH inhibits nodulation only by inhibiting rhizobial growth, for in *Pisum* and *Medicago*, at least, low pH inhibits nodulation even if rhizobial numbers on the root are very large (Lie, 1969, 1974, Munns, 1968).

Rhizobial colonization and survival in conventional media or in bulk soil is one thing: colonization of the host rhizosphere in acid media is another. There are few relevant observations. Some data on

clover rhizospheres in acid soil indicated the presence of appreciable numbers of rhizobia, which increased along with improvement of nodulation when calcium carbonate was added (Locs and Louw, 1965, Rovira, 1961). Likewise, in solution grown *Medicago* and *Pisum* rhizobial growth on the root or in root-washings increased as the solution pH was raised in the range critical for nodulation of these species (i.e. pH 4.5 to 5.5) (Lie, 1969, Munns, 1968).

Calcium requirements are low, of the order 10 μM, for the growth of all rhizobia so far tested in simple media (Bergersen, 1961, Norris, 1959, Steinberg, 1938, Vincent, 1962 a,b). Requirements for magnesium are higher, and in addition there is a large non-specific requirement for alkaline earth cations (Vincent, 1962a). The low calcium requirement is generally taken to mean that when calcium deficiency limits nodulation, it does not do so by limiting growth of the rhizobia. However, Lowther and Loneragan 1970 have shown that at moderately low calcium concentrations, multiplication of *R. trifolii* in the rhizosphere of *Trifolium subterraneum* can be inhibited enough to explain poor nodulation. Their data might imply that requirements in the rhizosphere are different than in the medium at large, or that there are special needs for calcium to ensure proper organization of the rhizobial population at potential sites of infection (see also Lie, 1969). Or, more simply, depletion of free calcium by the plant could make the rhizosphere deficient for rhizobia even when the bulk medium is not. This explanation needs no postulate that the rhizobial calcium requirement is different in the two phases.

Manganese toxicity tolerance of those few rhizobia studied is so large (Wilson and Reisenauer, 1972 a,b), that manganese toxicity would seem unlikely to inhibit their growth or survival in acid soils. However, there is little information on manganese concentrations actually present in soil solutions in the field moisture range. In certain Irish and British pastures, manganese toxicity, or soil acidity, leads to dominance of strains ineffective with introduced *Trifolium repens*, according to studies of isolates made from nodules (Holding and Lowe, 1971, Lowe and Holding, 1970, Masterson, 1968, Masterson and Sherwood, 1970). These data do not exclude the possibility that the soil acidity condition interferes with the ability of the *Trifolium repens* to nodulate selectively with effective strains (Masterson and Sherwood, 1969).

Effects of aluminum on rhizobial growth have not been tested. To do so would require maintaining a combination of low phosphate and adequate pH-buffering at low pH, in order to maintain effective concentrations of free aluminum ion. This same requirement holds for plant experiments on aluminum toxicity (Munns, 1965). For some legumes, putative effects of aluminum on rhizobia would require a pH too low to permit nodulation, but in legumes capable of nodulating at low pH, effects of aluminum on rhizobial growth might have important influence.

NODULATION AND NODULE FUNCTION

Effects of calcium and acidity on nodulation have been studied in

artificial media with several legumes. These include *Trifolium* species (Andrew, 1967, Loneragan and Dowling, 1958, Lowther and Loneragan, 1970, Small, 1968), *Medicago* species (Andrew, 1976, Munns, 1968, 1970), *Glycine max* (Albrecht, 1937), *Pisum sativum* (Lie, 1969) and *Macroptilium lathyroides*, *Lotononis bainesii*, *Stylosanthes humilis*, *Desmodium uncinatum*, and *Glycine wightii* (Andrew, 1976). In *Medicago sativa*, *Pisum sativum* and *Trifolium subterraneum* inhibition by low calcium or pH was partly offset when the size of inoculum was increased. This implication that part of the adverse effect on nodulation is due to inhibition of rhizobial growth is consistent with attempted rhizosphere counts (see previous section). However, increasing the inoculum size is effective only up to a point: even with very large inocula, acidity and low calcium can prevent nodulation by interfering with the infection process over and above effects on rhizobial colonization of the root surface (Lie 1969, 1974, Munns 1968).

The part of the infection process which is sensitive to calcium and acidity has not been identified. In *Medicago sativa* it coincides with curling of root hairs (Munns, 1968). Lowther and Loneragan (1970) suggested that calcium influences a postulated organization or congregation of rhizobia at potential sites of infection, reflected only indirectly in gross counts of rhizobia on the root surface. (Lie 1974).remarks on possible effects of pH on development or structure of the mucigel layer, in which rhizobial colonization of the root surface presumably takes place. I have suggested that both calcium deficiency and acidity diminish the association of calcium ions with cell walls, cell membranes, or enzymes involved in infection (Munns 1970, 1976). None of these suggestions is encumbered by firm data.

Some, but not all, of the above experiments show interactions between calcium and pH as illustrated in Figure 3. The adverse effects of acidity on nodulation are mitigated by high calcium, and the adverse effects of low calcium are mitigated by high pH. This happens also for plant growth, in legumes and non-legumes (Epstein, 1972). It is consistent with the well established inhibition of calcium uptake by acidity (Epstein, 1972), and also with the need for charge sites to be deprotonated before they can adsorb calcium. Species in which a calcium x pH interaction has been shown in simple media include *Glycine max* (Albrecht, 1937), *Glycine wightii* (Andrew 1976), most of the *Trifolium* and *Medicago* species studied (Andrew, 1976, Loneragan, 1973, Munns, 1970, 1976, Robson, 1969, Small, 1968), and *Stylosanthes humilis* (Andrew, 1976). Soil experiments, despite the difficulty of raising calcium availability or pH without influencing several other variables, have yielded results consistent with a calcium x pH interaction for *Trifolium* species (O'Toole and Masterson, 1968, Vincent, 1965), *Medicago sativa* (Munns, 1965), and *Glycine wightii* (Lee and Wilson, 1971).

In no case has magnesium been found to substitute for calcium in its effect on nodulation; nor has magnesium deficiency yet been found to inhibit nodulation or nitrogen fixation directly, despite its essentiality for growth of both host and rhizobia.

The range of pH and calcium in which nodulation responds varies, depending on host species and rhizobial strain. Table 2 is an attempt

Table 2. Relative Acid Tolerance of Legume Species*
(A First Approximation)

Species	Nodul'n	Growth†	Species	Nodul'n	Growth†
Acacia mellifera	2	?	*Medicago sativa*	1	1
Arachis hypogea	4	4	*Medicago truncatula*	1	1
Centrosema spp	4	4	*Medicago scutellata*	1	1
Coronilla varia	0	0	*Phaseolus vulgaris*		
Desmodium canum	3	3	black-seeded	3	3
Desmodium intortum	3	3	other	2	2
Desmodium uncinatum	4	3	*Pisum sativum*	2	2
Glycine max	3	3	*Pueraria* spp	?	5?
Glycine wightii	2	2	*Stylosanthes humilis*	5	4
Indigofera spicata	4	4	*Trifolium africanum*	4	3
Leucaena latisiliqua	4?	0	*Trifolium pratense*	1	2
Lotononis bainesii	5	4	*Trifolium repens*	1	2
Lotus corniculatus	2	2	*Trifolium subterraneum*	2	3
Macroptilium lathyro	5	5	*Trifolium ruepellianum*	3	3
Macrotyloma axillare	4	2	*Trifolium semipilosum*	3	3
			Vigna unguiculata	5	4

* rating scale from 0 (most intolerant of soil acidity) to 5 (most tolerant)
† growth when dependent on nitrogen-fixation

to rate some of the legumes according to their tendency to suffer from soil acidity conditions. Lest the tabulation be taken too seriously, it is my largely subjective attempt to compare results from different experiments done under different conditions. Most of the species are represented in only one trial. The ratings could change with inoculant strain or rate, with method of inoculation, or with the differences in soil or other medium. The table does not clarify where calcium deficiency or acidity is the major stress; and I had difficulty weighting the relative importance of critical levels on one hand versus the severity of effects on the other. There are real differences, however: cowpeas can flourish where alfalfa struggles and *Coronilla varia* dies. The table implies that tolerance ratings in terms of nodulation need not coincide with ratings in terms of growth and nitrogen fixation. This discrepancy is probably real (see below).

The nodulation process has been thought to need higher calcium and pH than is needed for nitrogen fixation or growth of plants with already established nodules (Jensen, 1942-3, Loneragan, 1973, Munns, 1976, Vincent, 1965). This implies that nodulation is the critical point at which these soil-acidity stresses impinge on the symbiosis. "Once you've got nodules in an acid soil, you've got it made." This "classical situation" (for lack of a less presumptuous label) is represented in Figure 4A. It may prove to be a special case, found in some species under certain greenhouse conditions. It has long been difficult to

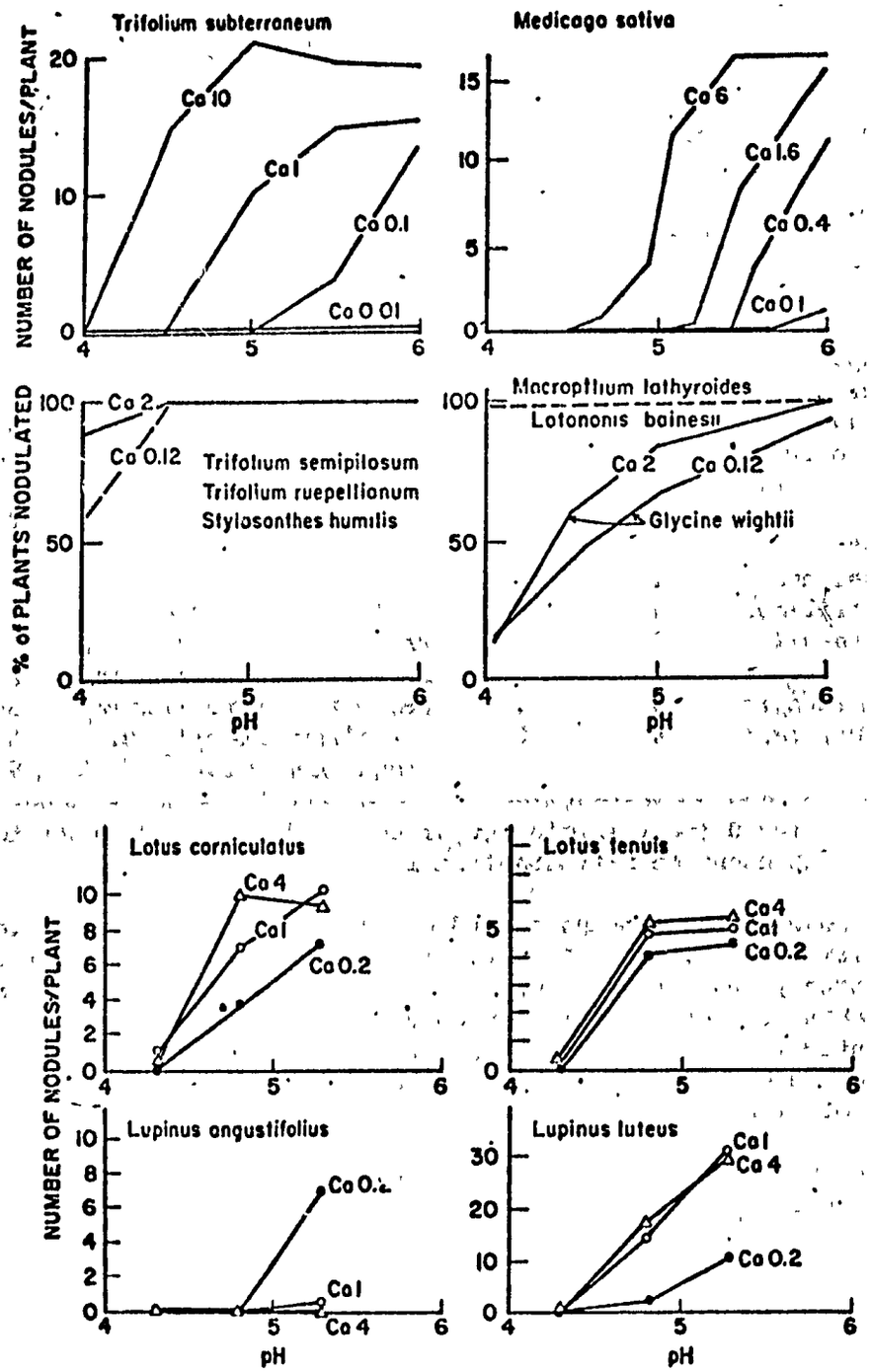


Figure 3. Effects of Ca and pH on nodulation of some legumes in solution and sand cultures. In most cases high Ca has moderated the inhibitory effect of low pH, but note (a) lack of either pH or Ca effect on *Macroptilium* and *Lotononis* (b) lack of Ca effect on *Lotus tenuis* (c) adverse effect of high Ca in *Lupinus angustifolius*. The graphs are schematic representations of data from Loneragan and Dowling (1958) for *Trifolium subterraneum*; Munns (1970) for *Medicago sativa*; Munns (unpublished) for *Lotus* and *Lupinus*; and Andrew (1976) for other species.

reconcile with agronomic evidence that legumes (e.g. soybean and alfalfa) sometimes respond to lime rates well above those that should be ample for good nodulation. These responses have had to be explained by supposed improvements in the soil's status of molybdenum, manganese, phosphate, sulfur, etc. But recent evidence shows that pH and calcium requirements for optimal nodule function can in fact be higher than for the establishment of nodules.

By raising the concentration of calcium in solution cultures, Banath *et al.*, were able to increase the growth and total nitrogen fixation of nodulated plants of *Trifolium subterraneum,* without altering the nodule weight per plant (Banath *et al.*,1966). The effect was small, and the case isolated. However, more recently, Andrew (1976) found that nodulated plants of several species responded to increase in either calcium or pH, implying that levels insufficiently low to stop nodulation still limited either the growth capacity of the host or the nitrogen-fixing function of the nodules. In a field liming trial with 18 species Munns, Fox, and Koch (1977) found that the majority of lime responses included an increase in rate of acetylene reduction per unit nodule weight, and that in only one species, Coronilla *varia,* could plant response be explained simply by increase in nodule number. *Macrotyloma axillare* presented the clearest "non-classical" picture (Figure 4B). At the seedling stage (three weeks), it had nodulated well at all lime rates, but the highest rates had already given a threefold increase in acetylene reduction per unit nodule weight and a small increase in plant dry weight. By harvest time, 10 weeks later, lime had caused a fivefold increase in plant weight and a sixfold increase in yield of nitrogen. The lime requirement for optimal dinitrogen-dependent growth was far above the requirement for good nodulation.

These recent experiments might differ from the earlier ones mainly in having provided better conditions for growth and nitrogen fixation (e.g. better light, water, soil temperature). Figure 4C shows how the "classical" situation could depend on the existence of a growth limitation, whose alleviation could then lead to the situation found in the recent experiments. The latter may be the more common, or at least the more representative of highly productive systems.

The physiological nature of acid and calcium effects on nodule function remains unexplored. Both factors might operate by causing calcium deficiency within the nodule. The role of calcium in the nodule could be the same as one of its main roles in the plant; namely, controlling the integrity of membranes. For example, in plant roots, moderate calcium deficiency (0.5mM external in barley) leads to leakiness of the plasmalemma (Epstein, 1972), which is closely related to the membrane envelope that surrounds bacteroids.

CORRECTION OF ACID SOILS FOR LEGUME GROWTH

In all but the most weakly buffered soils, raising the pH from the region 4-5 to the region 5-6 is likely to require something like 1000 to 5000 kg of lime/hectare. These high rates are necessary to

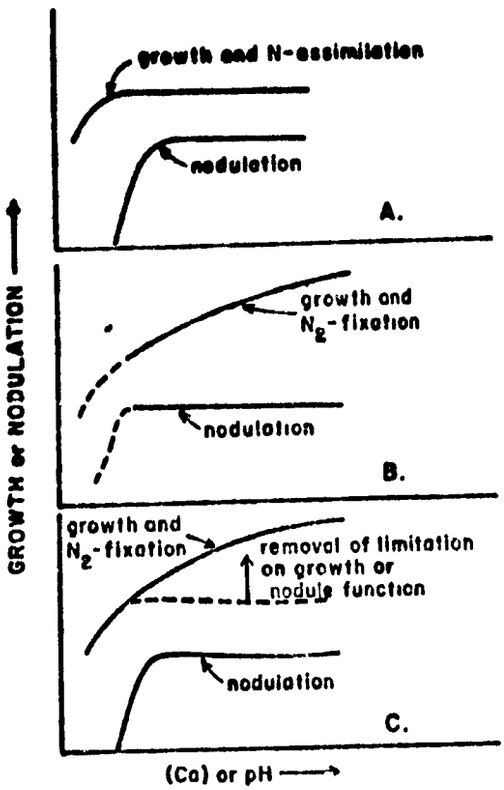


Figure 4. Sensitivity of nodulation to low Ca and pH, as compared with sensitivity of nodule function and plant growth (schematic).
A. "Classical" situation -- nodulation more sensitive than nodule function or plant growth.

B. Situation found in some recent experiments -- nodule function and plant growth inhibited although Ca or pH ample for nodulation. Andrew (1976); Banath *et al* (1966); Munns *et al* (1977).

C. Representation of argument that A is special case of B, observed under conditions unfavorable to optimal nitrogen fixation or plant growth.

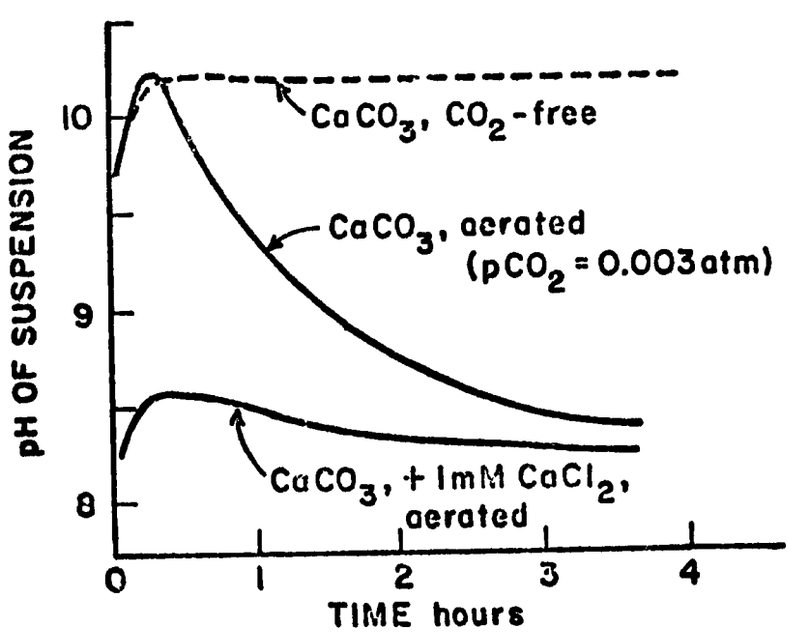


Figure 5. Time change of pH following addition of reagent calcium carbonate to water (5% suspension). Effects of carbon dioxide and addition of Ca^{+2}. (From experimental data of V.W. Fogle).

correct effects of acidity, aluminum toxicity, or manganese toxicity sufficiently for the growth of sensitive plant species (Pearson, 1975, Pearson and Adams, 1967). On the other hand, just to correct calcium deficiency may require much less lime (Loneragan, 1973, Munns, 1976, Pearson, 1975). An example is recent CIAT trials in which yields of *Vigna, Stylosanthes, Centrosema* and *Desmodium* were maximized by 100 kg lime/hectare (Pearson, 1975, Spain *et al.*,1974). The low rates of lime needed in this case could easily be economic, and at the same time could easily be overshot experimentally by the ton or so of lime commonly added in lime trials.

Calcium deficiency is not always corrected by small amounts of lime. In a Hawaiian Oxisol, high in manganese, some legumes responded to lime rates up to 10 or even 20 tons/hectare, mainly because of increased availability of calcium (Munns and Fox, 1976). In this soil, calcium in solution was initially very low at 0.3 me/liter, and increased little until the lime rate exceeded 8 tons (pH 5.8). Other soils with large pH-dependent charge may similarly buffer calcium at extremely low concentration in solution (Munns, 1976, Munns and Fox, 1977). I have recently found this property in 7 out of a group of 12 acid northern California soils.

The amount of lime can sometimes be cut by placing lime with the seed or applying it as a seed pellet (Loneragan, 1973, Munns, 1976, Vincent, 1965). (Note that the benefits of lime pelleting need not be due only to the lime. Neutral pelleting materials or adhesive alone are sometimes as effective). Modification of the seedling rhizosphere by drilling or pelleting can be completely effective where the difficulty is faulty nodulation during establishment and where manganese and aluminum toxicities are not severe. In other cases, pelleting alone, or even locally drilled lime, do not optimize production (Anderson and Moye, 1952, Loneragan, 1973, Loneragan *et al.*, 1955, Paton, 1960).

Both liming and lime pelleting can be injurious. Liming certain soils just to pH 6 has induced, in legumes, deficiencies of micronutrients (e.g. Anderson, 1970, Munns, 1976, Pearson and Adams, 1967, Ruschel *et al.*, 1967, Russel, 1966) and of phosphate (Barrow 1965, Fox *et al.*, 1964, Lowther and Adams, 1970). Further, some species may simply be adapted to low calcium and pH, and therefore nodulate and grow worse if these factors are raised to "normal" levels. This is supported by observations from sand and solution cultures with *Stylosanthes* and *Lotononis* (Andrew, 1976) and *Lupinus* species (D.N. Munns unpublished); and it may be true for other species from such genera as *Pueraria, Desmodium, Centrosema, Ornithopus, Lotus*, and *Vigna*.

The literature may give an exaggerated view of the agronomic importance of adverse effects of lime applied to soil. Most of the information is from short term experiments in greenhouses, but in Hawaiian field trials (Munns and Fox, 1976) lime-induced depressions of growth which appeared very significant early after emergence persisted only in *Stylosanthes guyanensis* and *S. fruticosa*. They gave way to positive lime responses in *Desmodium canum, D. intortum, Glycine sightii, Macrotylona axillare, Trifolium subterraneum*, and *Phaseolus vulgaris*.

Lime pelleting may suppress nodulation more commonly than would have been expected from the early research which developed the process for *Trifolium* and *Medicago* species. Pelleting with reagent calcium carbonate almost eliminated nodulation of *Lupinus* and *Ornithopus* sown into soils of pH 6 (Parker and Oakley, 1965). Norris (1971, 1972 a,b), found variable pelleting response in a range of tropical pasture legumes. In one trial, lime pelleting depressed early growth of *Lablab* (Norris, 1972a). In another trial, it improved nodulation of *Desmodium* if the seeds were sown immediately after pelleting; but if sowing was delayed, pelleting depressed nodulation because of a drastic decline in rhizobial numbers on the seed (Norris, 1972b). For *Stylosanthes guyanensis*, simple inoculation was found better than lime pelleting (Graham and Hubbell, 1975). Contact with lime can kill fast-growing as well as slow-growing rhizobia. In tests on inoculated seed of *Trifolium subterraneum*, several lime materials, including a reagent calcium carbonate, decimated rhizobial numbers (Radcliffe, *et al.*, 1967). None of the research explains why some lime materials injure some rhizobia. Simple alkalinity, which varies from one lime to another, seems a likely culprit. Even pure calcium carbonate is a fairly strong base. Suspended in water, it will raise the pH above 10 (Figure 5). The pH will drop to about 8.3 when the reaction with atmospheric carbon dioxide reaches equilibrium; but this equilibration can take hours, even in a dilute carbonate suspension vigorously bubbled with air. Thus coating with calcium carbonate, even without excess alkali, could treat the inoculant to a lethal exposure to alkalinity. The dissociation of calcium carbonate, and hence its alkalinity, is suppressed by a surplus of calcium ions. Perhaps accidental or deliberate contamination with a little neutral calcium salt helps to make lime safe for rhizobia.

SELECTION OF ADAPTED LEGUMES AND RHIZOBIA

The use of legumes and rhizobia that are adapted to soil acidity reduces the need for expensive inputs of lime. Some tropically important types, evolved or agriculturally developed in areas of neutral, alkaline, or calcic soil, probably contain many types unadapted to acid soil conditions. Legumes and rhizobia that have been evolved or developed against a background of soil acidity should have undergone some natural or accidental selection for acid tolerance, but the pressure of selection for vigorous nitrogen fixation might well have been weak.

Successful selection requires genetically determined differences that are large enough to matter and that are consistently expressed under different conditions of culture, and in this case, different types of soil acidity stress. One of the best examples is at the generic level - the high sensitivity of *Medicago* species as compared with *Trifolium subterraneum*. In soils and artificial media with varying degrees of molybdenum deficiency, calcium deficiency, aluminum toxicity, and manganese toxicity, subterranean clover consistently nodulates and grows

better than the medics (Anderson, 1970, Loneragan, 1973, Millikan, 1961, Munns, 1965, 1976, Robson and Loneragan 1970a,c, Vincent, 1965). Yet even in this case, the differences in acid-soil tolerance are moderated by other factors, being amplified when phosphate is deficient or free aluminum is high (Loneragan, 1973, Munns, 1965).

Certain tropical species have shown good tolerance of soil acidity in comparative trials. In a Colombian Oxisol, 20 varieties of *Vigna unguiculata* all nodulated and grew better at very low lime rates (0.5 ton/acre) than did 225 varieties of *Phaseolus vulgaris* (Spain *et al.*, 1974). In greenhouse trials with an extremely calcium deficient soil (Andrew and Norris, 1961), nodulation failed less spectacularly without lime in a group of tropical pasture species (*Desmodium, Indigofera, Centrosema, Stylosanthes* and *Macroptilium* species) than it did in some temperates (*Medicago* and *Trifolium* species). In recent sand culture experiments with well-controlled calcium and pH variables, Andrew (1976) found large differences in nodulation response, and in growth response of nodulated plants, approximately as follows: very sensitive: *Medicago sativa, M. scutellata, M. truncatula, Glycine wightii*. inhibited only below pH 5: -*Desmodium uncinatum, Trifolium repens* inhibited below pH 4.5: -*Stylosanthes humilis, Trifolium semipilosum, T. rueppellianum* not inhibited (pH 4.0 calcium 0.12 mM): -*Macroptilium lathyroides, Lotononis bainesii*. In a field liming trial on a Hawaiian Oxisol with low free calcium and high free manganese (Munns *et al.*, 1976, 1977), species varied greatly in patterns of nodulation and growth response (Figure 6).

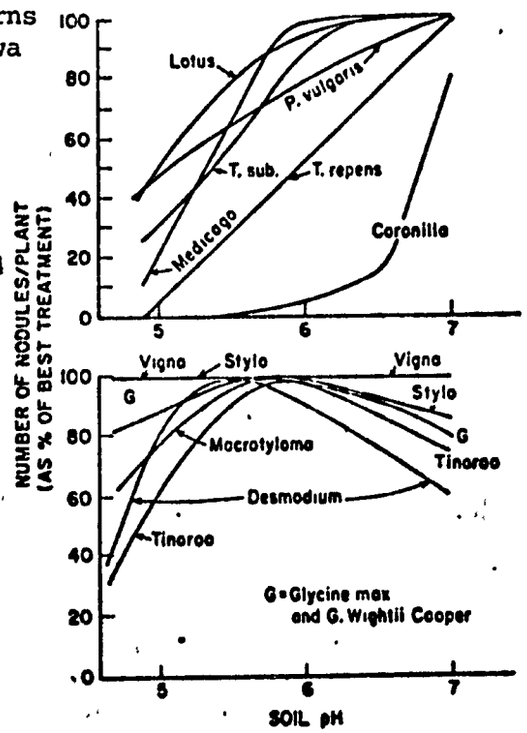
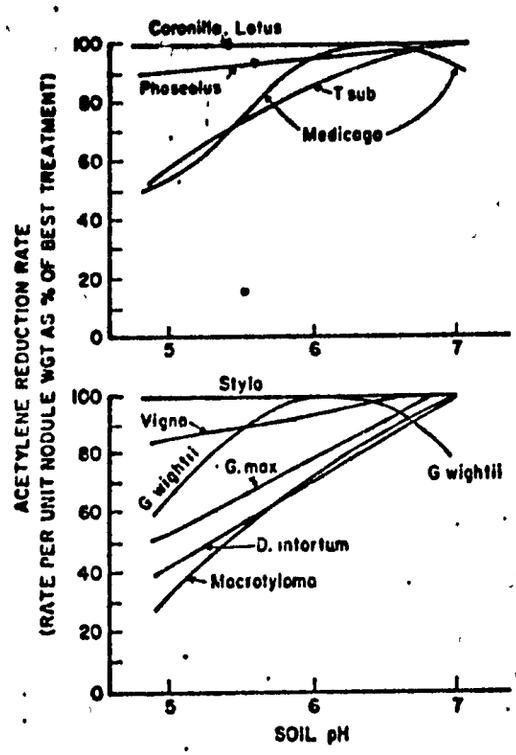
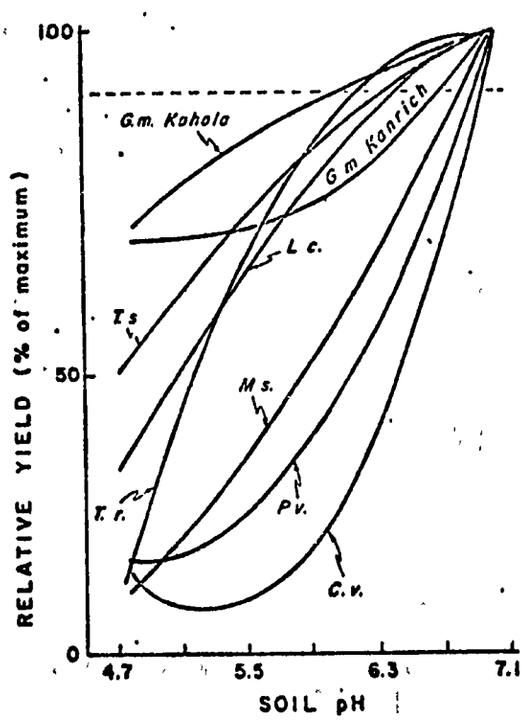


Figure 6 (A), (B), and (C). Some patterns of response to lime by legumes on Wahiawa silty clay at Poamoho, Oahu, Hawaii. (From data of Munns and Fox, 1976; Munns *et al* 1977).

A. Nodulation during early growth (2 - 4 weeks after emergence).



B. Acetylene reduction rate per unit nodule weight during early growth (intact root portions assayed in the field).



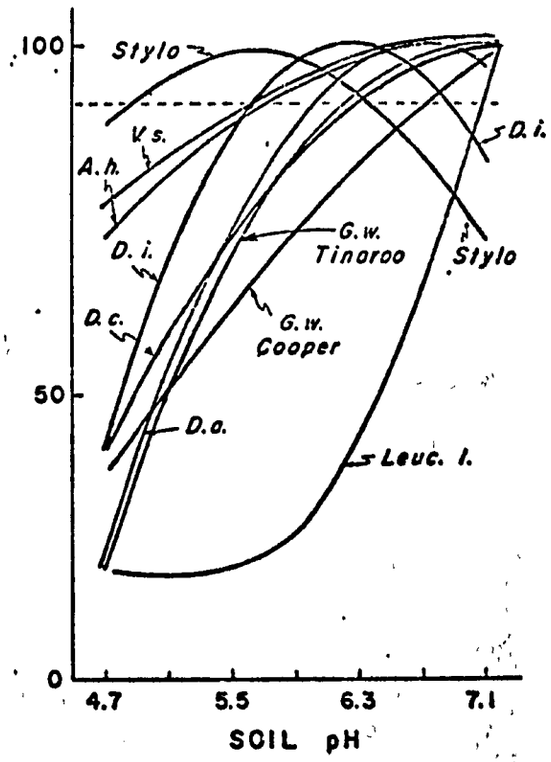


Figure 6 (C) *(Page 228, lower portion)*. **Some patterns of response to lime by legumes on Wahiawa silty clay at Poamoho, Oahu, Hawaii. (From Munns and Fox, 1976; Munns *et al* 1977).**

Harvest data, relative yield of fruit *(Phaseolus vulgaris)* or relative vegetative yield (other species).

Key to species identification:

Arachis hypogaea - - - - - A.h.
Coronilla varia - - - - - C.v.
Desmodium canum - - - - - D.c., Desmodium
Desmodium intortum - - - - D.i., Desmodium
Dolichos axillaris - - - - D.a., Macrotyloma
Glycine max - - - - - - - G.m., Khala, Kenrich
Glycine wightii- - - - - - G.w., Cooper, Tinaroo
Leucaena latisiliqua - - - Leuc.l.
Lotus corniculatus - - - - L.c.
Macrotyloma axillare - - - D.a.
Medicago sativa- - - - - - M.s.
Phaseolus vulgaris - - - - P.v.
Stylosanthes fruticosa - - Stylo
Stylosanthes guyanensis- - "
Trifolium repens - - - - - T.r.
Trifolium subterraneum - - T.s.
Vigna unguiculata - - - - V.s.

From these trials and others (e.g. Foster, 1970, Jones and Freitas, 1970, Olsen and Moe, 1971, Souto and Dobereiner 1968, 1969), tolerance of soil acidity appears dependable in *Vigna unguiculata, Macroptilium lathyroides,* and some *Stylosanthes* species. In *Arachis hypogea,* nitrogen fixation and vegetative growth are tolerant but there are reports that high calcium is needed for seed setting. By contrast with these species (and probably many others), some tropical species tolerate soil acidity no better than common temperate species. Examples are *Glycine wightii Macrotyloma axillare* and *Leucaena latisiliqua.*

Within species, there are varietal and ecotypic differences in tolerance of soil acidity factors. Examples are differences in tolerance of aluminum and manganese in *Melilotus alba* (Ramakrishnan, 1968), *Glycine wightii* (Souto and Dobereiner 1968, 1969), *Medicago sativa, Glycine max* and *Trifolium subterraneum* (Epstein, 1972, Millikan, 1961); and differences between ecotypes of *Trifolium repens* in calcium requirement and ability to compete in acid soil (Masterson and Sherwood, 1970, Snaydon, 1971). In *Phaseolus vulgaris,* a comparison of 125 black-seeded varieties with 100 non-black varieties showed that black-seeded varieties had superior ability to nodulate and produce at low levels of lime (Spain *et al.,* 1974). Within each major group of rhizobia, strains differ considerably in ability to survive and grow at low pH (Graham and Parker, 1964, Ham *et al.*,1971). Significant differences in ability to nodulate the host in acid soil have been found amongst rhizobia for *Medicago sativa* (Munns, 1965) and *Leucaena latisiliqua* (Graham and Hubbell, 1975, Norris, 1972b).

The probable importance of interactions involving both host and rhizobia genotypes is an obstacle to simple definition of tolerances of either the host or the *Rhizobium.* Norris (1971) found that different cultivars of tropical pasture species responded to lime pelleting differently with the one rhizobial strain, and that different strains responded differently with the one host cultivar. In our own trials (Munns *et al.,* 1976, 1977), *Coronilla varia* and *Leucaena latisiliqua,* though inoculated and perhaps nodulated by similar rhizobia (Nitragin M inoculant), responded differently to lime. High rates of lime improved growth of both species, but in *Coronilla* this was due to improvement in nodulation, whereas *Leucaena* nodulated well without lime but evidently needed high rates for the nodules to become fully effective. In the same trial, Nitragin EL inoculant produced good nodulation without lime on both *Vigna unguiculata* and *Macrotyloma axillare;* but *Macrotyloma,* unlike *Vigna,* needed lime for the nodules to become fully effective. Selection of symbionts for ability to nodulate under a stress is valueless unless the resulting nodules are effective under that stress.

By way of summary, comparisons and systematic screening of hosts and rhizobia for acid tolerance should take into account the following points:

1. there is little information on acid tolerances of many important legumes (including tropical grain legumes with the exception of *Vigna unguiculata* and *Phaseolus vulgaris*).

2. tolerance of one soil-acidity factor need not guarantee tolerance of another; the particular combination of pH, calcium, manganese and aluminum needs to be characterized, quantitatively whenever that should become possible.
3. successful nodulation under stress does not guarantee successful nitrogen fixation.
4. host - rhizobial interactions are to be expected.

These considerations make it certain that the characterization of useful acid-tolerant types will proceed slowly, given current empirical methods of comparison and screening. Simple, accurate, predictive criteria would help.

Taxonomy of the *Rhizobium* "species" is no such predictor. Reasonably acid-tolerant associations involve some of the cowpea miscellany, some *R.lupini*, some *R.japonicum*, and some *R. trifolii*. The criteria of slow growth or non-acid production may still have value for predicting ability to nodulate in acid soil, if not for predicting effectiveness. The point has not been sufficiently tested. Correlations have been shown between acid production and tendency to associate effectively with a particular host. But the main question, unanswered, is whether acid production correlates with symbiotic competence in acid soil. Norris's later trials (1971, 1972 a,b) would imply that the properties of acid production or slow growth are not completely accurate or precise indices of the need for lime or lime pelleting.

Any search for better predictive criteria starts, of course, with firm quantitative information on the acid tolerance of a range of host and rhizobia types. It could help if we better understood the ways in which soil acidity factors interfere with a diversity of symbioses.

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NUTRITIONAL RESTRAINTS FOR TROPICAL GRAIN LEGUME SYMBIOSIS

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ABSTRACT

The achievement of high levels of nitrogen fixation, and high yields, will only be reached when all nutrients essential for plant growth are readily available. Frequently legume plants relying on N_2 for growth are more demanding for proper nutrition than plants supplied with combined nitrogen. The toxicity of aluminum or manganese, soil pH, nitrogen, phosphorus and molybdenum are some of the elements that may specifically limit the efficiency of the symbiosis in the tropics.

Phosphorus deficiency has been found to be the main limiting nutrient to legumes in the tropics. Fertilization with phosphorus in acid soils often is complicated by the high capacity of the soil to fix this element.

Because of the need to increase grain yield production to meet world demands and the short cycle of some of the tropical legumes, supplementation with mineral nitrogen might be necessary. Several conflicting results found with *Phaseolus vulgaris* and *Glycine max* will be presented and the interactions of N_2 fixation with combined nitrogen fertilizer explored. A few data using a more basic approach by following up the nitrate-reductase and nitrogenase activities after nitrogen fertilization will be presented.

To allow plants to show full potential in grain production, once aluminum or manganese toxicity and phosphorus deficiency have been eliminated, one needs to look at the micronutrient deficiencies. Promising results have been found by applying fritted trace elements (F.T.E.) directly in soil or by pelleting the seeds of perennial legumes. *Phaseolus vulgaris* seems to require specific Mo applications, especially in acid soil. This element in connection with liming increased bean yield by 913 percent.

INTRODUCTION

Dry bean *(Phaseolus vulgaris)* is the most important grain legume in Latin America, peanut *(Arachis hypogea)* in Africa and soybeans *(Glycine*

max), peanuts, pigeon pea *(Cajanus flavus)* and chickpea *(Cicer arietinum)* in the far East (F.A.O. 1970, Auckland and Singh, 1976). Cowpea *(Vigna sinensis)* is next to peanuts in Nigeria (Dawson, 1970) and substitutes for dry beans in the northern part of Brazil (Krutman, 1968). Soybean *(Glycine max)* is the major protein source for animals and humans; successful breeding programs are extending it into tropical areas.

It seems that soil acidity, together with aluminum and or manganese toxicity, deficiency of phosphorus and molybdenum, are some of the factors that may limit grain yield of tropical legumes dependent on symbiotic nitrogen for growth. More emphasis will be given to dry beans and soybeans in view of their importance for Latin America and because they are the subject of our work in Brazil.

Potassium deficiency has been omitted because it is a minor problem in most tropical soils (Table 1) and does not have a specific role in the symbiosis.

PHOSPHORUS AND SULPHUR

The vital role played by phosphorus in reactions involving energy transfer, and more specifically, ATP in nitrogenase activity, suggests that plants dependent on symbiotic nitrogen for growth may require more phosphorus than those dependent on combined nitrogen. Sulphur is part of the nitrogenase molecule and is very important in the nitrogen metabolism in the plants.

Van Schreven (1958), Andrew (1962), Vincent (1965), Dart (1974), Graham and Hubbell, (1974) and Gibson (1975) reviewed the effect of phosphorus on legume symbiosis. It is worthwhile to include here results of Goepfert and Freire (1969) with soybean indicating an indirect effect of high doses of phosphate decreasing available aluminum and manganese in the soil, and lessening their toxic effect on the plants.

The acid soils in the tropics are phosphorus deficient. In Table 1 the results of a number of NPK experiments with dry beans are summarized. Most striking is the effect of phosphorus. The soils formed from volcanic material generally show low phosphorus content. The adsorption of this element in acid conditions in the presence of iron, aluminum oxides and clay particles increases the difficulty of supplying phosphorus to the plants. To saturate the soil's P-fixing capacity, large amounts of fertilizer are necessary which are too expensive for the small farmer. The use of species or varieties better able to extract phosphorus under these specific conditions or to increase phosphorus availability by using vesicular arbuscular (VA) mycorrhizas as demonstrated by Crush (1974) has as yet not been well studied. This author observed that *Stylosanthes* showed fewer root hairs and benefited most from the VA mycorrhiza by extracting phosphorus from a soil with low levels of this element. It was also shown that VA mycorrhiza enhanced phosphorus uptake and improved host growth in many species (Mosse, 1973) including soybean (Ross and Harper, 1970; Ross, 1971 and Jackson, *et al.*,1972). The fact that *Stylosanthes* shows less root hairs, is tolerant to acid soil with moderate aluminum and manganese toxicity and is more efficient

Table 1. Effect of nitrogen, phosphorus and potassium on *Phaseolus vulgaris* yield-Number of experiments with increase or no increase due to fertilizer.

Nitrogen		Phosphorus		Potassium		Grain*	Reference
Increase	No increase	Increase	No increase	Increase	No increase	(kg/ha)	
2	0	-	-	-	-	1,380	Mascarenhas, *et al.*, 1966
5	2	7	0	0	7	1,253	Miyasaka, *et al.*, 1966/a
3	1	3	1**	1	3	2,250	Miyasaka, *et al.*, 1966/b
1	3	3	1**	0	4	1,550	Miyasaka, *et al.*, 1966/c
0	8	3	5	1	7	-	Miyasaka, *et al.*, 1967
2	1	3	0	1	2	pot	Martini and Pinchinat, 1967
0	2	2	0	0	2	793	Mascarenhas, *et al.*, 1967
3	1	4	0	0	4	1,112	Mascarenhas, *et al.*, 1967/b
-	-	1	0	-	-	pot	Goepfert, 1970/a
1	0	1	0	-	-	1,718	Goepfert, 1970/b
1	0	-	-	-	-	pot	Guss e Dobereiner, 1972
1	0	1	0	-	-	735	Miller, *et al.*, 1973
1	1	2	0	-	-	762	Guazzelli, *et al.*, 1973
1	0	1	0	-	-	875	Almeida, *et al.*, 1973
2	6	6	2	0	8	2,201	Kranz, *et al.*, 1976
0	1	1	0	-	-	2,384	Kornelius, *et al.*, 1975
1	0	-	-	-	-	- -	Franco, (results not published)
24	26	38	9	3	37		

* Highest yield in the experiment.

** Sites previously fertilized with phosphorus.

in extracting micronutrients from the soil (De-Polli *et al.*,results not published) indicates adaptation to the acid soils in the tropics. Greater benefit from mycorrhizal association and selective assimilation of nutrient through microorganisms support this.

Care must be taken in using triple superphosphate to cover phosphate needs because in many situations sulphur is also deficient in tropical soils (McClung *et al.*,1968 and Mascarenhas 1967/a). The former authors found an increase of 37 percent in soybean grain yield with sulphur fertilization.

The use of a mixture of rock phosphate with sulphur has shown encouraging results (Swaby, 1974 and Lopes, 1975). Bromfield (1975) observed similar yield of peanut with fertilization with simple superphosphate and application of a mixture of rock phosphate and sulphur. The use of sulphur oxidation and phosphorus solubilization for annual legumes is confined to areas with high temperature and high humidity (Burns, 1967). However the benefit to the following crop has not been well studied.

CALCIUM AND MAGNESIUM

Calcium is more important for root hair infection than for bacterial growth (Norris, 1958; and Munns, 1970). At low pH more calcium is required than at high pH (Munns, 1970); this however varies with the plant. The tropical legumes seem to require less calcium than the temperate ones (Norris, 1958). Magnesium is required for bacterial growth and is necessary for ATP binding in nitrogenase activity.

The calcium and magnesium supply are generally associated with liming the soil to eliminate aluminum and manganese toxicity, by using dolomite lime; it will be covered elsewhere in this workshop.

COMBINED NITROGEN

Combined nitrogen may affect the legume symbiosis in several ways. The application of NO_3^- inhibits nodule formation and growth (Wilson, 1917; Nutman, 1956 and Raggio *et al.*,1957) and nitrogenase activity.

Hinson (1975) claims that NO_2^-, the product of NO_3^- reduction, inhibits indoleacetic acid (IAA) production, which is necessary for nodule initiation (Nutman, 1963). However, A.H. Gibson, (personal communication) observed that the application of nitrate delayed the first nodule appearance in plants inoculated with mutants of *Rhizobium* strains lacking nitrate reductase as much as that in plants inoculated with the parent strain with nitrate reductase. This is an indication that the nitrate in the medium produced by the bacteria is not responsible for delaying nodulation.

Ammonium inhibits nitrogenase synthesis and activity. However an initial nitrogen input is necessary for plant growth, nodule formation and symbiosis establishment. There are several observations to show that an application of a small amount of nitrogen may increase nodulation at flowering and final yield (Pate and Dart, 1961; Kapusta *et al.*,1970;

Guss and Dobereiner, 1972; Gibson and Nutman, 1960; Dart and Mercer, 1965 and Gates and Wilson, 1974). Munns (1968) showed that 0.02 to 0.05 mM N inhibits nodulation at the beginning but not in later stages of the plant. Similar results were obtained with cowpea by Dart and Mercer (1965).

Many references in the literature stating that the symbiotic process in soybeans supplies inadequate nitrogen for high yield are based on the conclusions of Norman (1944), Norman and Kranpitz (1945), Thornton (1946) Allos and Bartholomew (1959) and Hardy *et al.*,(1971). More recently results of Huxley *et al.*(1976) supported this idea. Allos and Bartholomew (1959) based their conclusion on total weight of 10-week-old plants grown in solution culture. Norman and Kramptiz (1945) reported data from four greenhouse experiments, in which plants were grown for varying time up to 96 days. All conclusions on N response were based on plant-weight data. Thornton (1946) reported N responses for plant weight and seed weight but his seed weight data were from 11-week-old, immature greenhouse plants. The conclusions of Norman (1944) were based upon results of plants not well nodulated and with low production. Hardy *et al.*,(1971) with experiments in the field during three seasons, concluded that only 25 percent of nitrogen was supplied by the symbiosis. However they did not show any data on grain yield or total plant nitrogen. Their conclusions were reached relying entirely on determination of acetylene reducing activity throughout the plant cycle. Also it is not known whether nitrogen fertilizer in these experiments would have increased grain yield. The results of Huxley *et al.*, (1976) were from plants grown in controlled conditions and the great benefit of mineral nitrogen was up to flowering. The seed production was little affected. Bhangoo and Albritton (1975) observed a yield increase of 500 kg/ha by adding 400 kg N/ha.

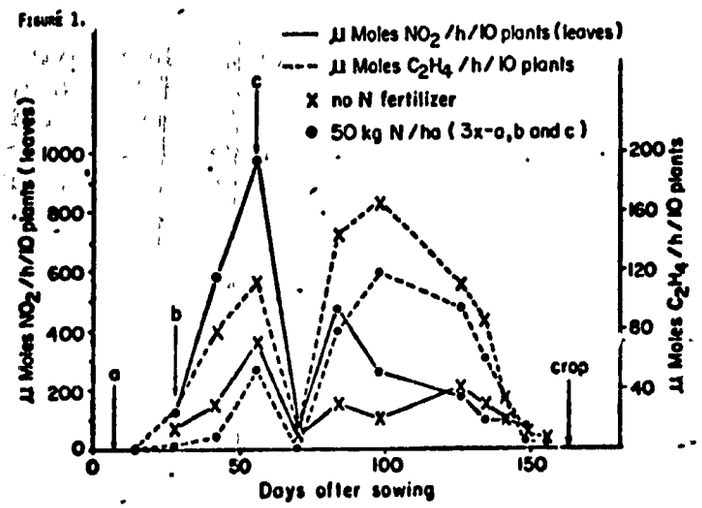

Figure 1. Nitrogenase and nitrate-reductase activity in soybean throughout the growth cycle. Each point is a mean of 6 blocks.

The results presented in figure 1 and that of Thibodeau and Jaworski (1975) indicate that the contribution of the symbiosis is more significant after flowering, when the nitrogenase is more active and the assimilation of nitrate is much less. Weber (1966) over a 6 year period did not obtain statistically significant increases in seed yield of nodulated soybeans with increments of N up to 168 kg/ha. His experiments were conducted at the same area where Norman (1944) got increases by adding nitrogen. However the average yield obtained by Weber (1966) was more than double the yield reported by Norman. Most recent papers do not show any significant increase of yield in well nodulated soybeans with nitrogen application up to 400 kg per hectare (Williamson and Diatloff, 1974; Hinson, 1975; Summerfield *et al.*,1975; Hathcock, 1975; Olsen *et al.*,1975 and Pal and Saxena, 1975).

Results of Bhangoo and Albritton (1975) and Pal and Saxena (1975) indicate that heavy doses of nitrogen are necessary for non-nodulating isolines to reach yields of well nodulated isolines. This could be explained by postulating that non-nodulating soybean plants require more energy for the greater proliferation of roots, and for uptake and reduction of nitrate N, then is required by nodulated plants for the symbiotic reduction of N_2 to amino-N (Weber, 1966). Results from Thibodeau and Jaworski (1975) and Franco *et al.*,(1976) indicate that the nitrate reductase activity peaks at flowering and decreases very rapidly at early pod filling stage, while nitrogenase peaks at early pod filling stage with a less rapid decline of activity (see also Hardy *et al.*,1971). At the same time the soybean nodules show a specific nitrate reductase activity greater than the specific activity of the leaves. It averages 10 percent of the total activity in leaves plus nodules, during the whole plant cycle. It has not been proved that this is an assimilatory process of nitrogen from the soil, but it may be an explanation why the non-nodulating isolines need so much nitrogen to match yields with the nodulated isolines.

The literature shows more conflicting results with dry bean than with soybean. Guss and Dobereiner (1972) observed, in greenhouse experiments, that the application of 23 ppm of nitrogen at sowing and 20 days after, increased nodule number and nodule weight at flowering. They found a great variation between *Rhizobium* strains in ability to nodulate in presence of nitrogen. Table 2). Franco and Dobereiner (1967) observed differences between cultivars of *P. vulgaris* in nodulation in the presence of small amounts of nitrogen, the cultivar Rico 23 being the most affected. Miyasaka *et al.*(1963) also found that small amounts of nitrogen applied at sowing increased grain yield, but no increase resulted from application at flowering. Bazan (1974) reviewed the effect of nitrogen on dry bean in Central America.

A survey of experiments where nitrogen was applied to *P. vulgaris* is included in Table 1. Twenty four of 50 experiments, conducted under several conditions, showed increased dry bean yield with nitrogen fertilization. In some of these experiments the yield was quite low, and in most of them no care was taken to obtain good nodulation; in a few cases there was no inoculation with *Rhizobium*. Kornelius *et al.*,(1975/a) reported no response to applied nitrogen with an average grain yield of

Table 2. Differences between ability of *Rhizobium* strains to nodulate *Phaseolus vulgaris* in the presence of combined nitrogen (Means of 3 replicates).*

Rhizobium strain	N added (20 ppm/pot)	Total plant N (mg/pot)	nodule/pot number	nodule/pot dry weight (mg/pot)
F-413	Control	19.4	277	110
	sowing	45.5	270	179
	20 days	34.8	330	136
F-300	Control	21.3	270	112
	sowing	57.4	263	186
	20 days	35.8	278	164
F-301	Control	21.1	305	149
	sowing	57.7	337	253
	20 days	31.6	272	155
F-304	Control	18.3	365	143
	sowing	69.6	406	307
	20 days	69.6	418	333
F-56	Control	23.9	74	29
	sowing	38.4	68	50
	20 days	31.9	1	1
F-207	Control	10.1	37	15
	sowing	75.7	197	207
	20 days	45.5	33	28
F-310	Control	13.6	993	160
	sowing	62.6	423	250
	20 days	36.1	527	277
F-33	Control	11.1	32	17
	sowing	25.2	243	134
	20 days	32.1	375	194
F-267	Control	11.6	53	33
	sowing	46.2	173	152
	20 days	36.2	205	146
F-403	Control	13.1	4	20
	sowing	25.5	0	0
	20 days	47.8	106	130

* Extracted from Guss and Dobereiner (1972).

Table 3. Effect of nitrogen fertilizer on grain yield of dry bean *(Phaseolus vulgaris)*. (Mean of 4 replicates).

N (kg/ha) applied at/:			Grain yield
sowing	15 days	30 days	kg/ha
0	0	0	2 300
Inoculated with *Rhizobium*			2 398
30	0	0	2 217
0	30	0	2 231
0	0	30	2 247
10	20	0	2 338
20	10	0	2 199
20	20	20	2 157
20	40	0	2 240
0	40	20	2 338

No significant difference.
(Extracted from Kornelius *et al.*,1975/a).

2,276 kg/ha in field experiments (Table 3). Kranz *et al.*, (1975) also reported no response to nitrogen in several experiments in one of the best bean growing areas in Brazil. Kornelius *et al.*,(1975/b) did not observe final seed yield increases by applying nitrogen with phosphate fertilization. However when no phosphorus was applied, the addition of 30 kg N/ha increased the grain yield by 32% indicating a specific effect of phosphorus on nitrogen fixation.

Because of the short growth cycle of this legume, a supplement with small amounts of nitrogen might increase yields. The conflicting results described indicate that a more basic approach is necessary, such as following the assimilation of nitrogen from both sources (soil and air) after nitrogen fertilization, instead of just looking at yield. Figs. 2 and 3, show a preliminary experiments in this direction.

Results obtained by Summerfield *et al.*, 1975 with cowpea *(Vigna sinensis)* indicated that it was impossible to increase significantly the seed yield, above that obtained from nodulated plants receiving a very low level of combined nitrogen. Indeed nodulated plants grown without any combined nitrogen yielded only 10% less seed than non-nodulated supplied with 240 ppm N throughout their growth.

MICRONUTRIENTS

Evans and Russell (1971), Munns (1976) and Gibson (1976) have reviewed the effect of the minor element on the symbiosis. Molybdenum is a component of nitrogenase (fraction I), Iron of nitrogenase (fractions I and II) and of leghaemoglobin. Boron is important for the vascular

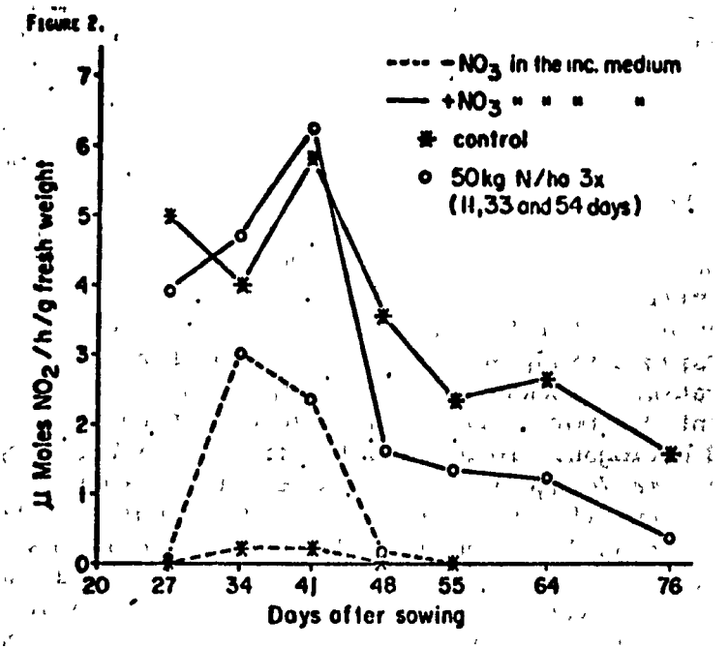

Figure 2. Nitrate-reductase activity in the third trifoliate leaf of *Phaseolus vulgaris* from early growth to decay. Each point is a mean of 3 replicates.

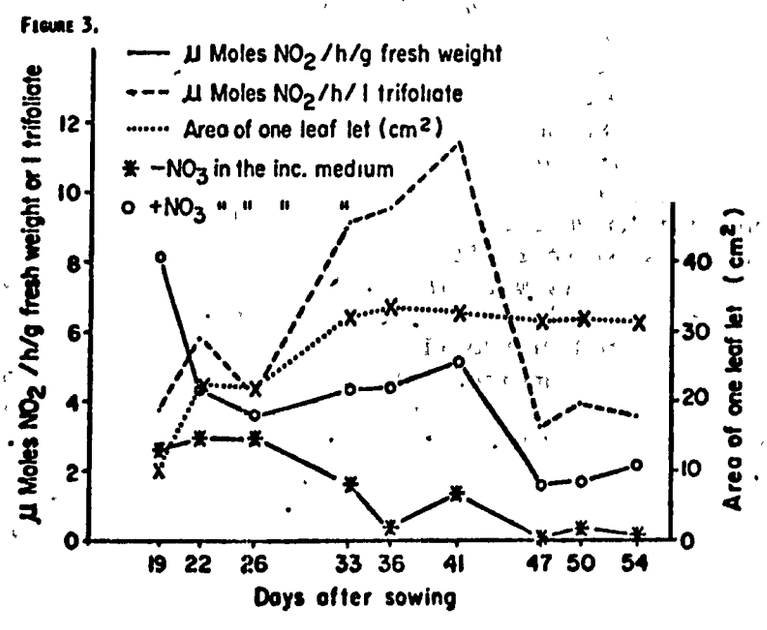

Figure 3. Total nitrate-reductase activity of *Phaseolus vulgaris* trifoliate leaf, and specific activity correlated with leaf expansion. Each point is a mean of 3 replicates.

tissue of the nodules. Copper affects bacterial growth and activity (Cartwright and Hallsworth, 1970). A requirement for cobalt has been shown; it is involved in cobamide coenzymes that abound in normal nodules (Evans and Russel, 1971).

There is a big difference between the demonstration of a minor element need for the symbiosis in pots and in the field, where several requirements, besides the presence of the nutrient, are to be met before a response is obtained. Several experiments fail to show response to a micronutrient, because the level of production is low with a major factor limiting the yield. Ruschel and Eira (1968) did not get any molybdenum benefit in soybean until they limed the soil to eliminate toxic available manganese. Using a sandy soil Franco and Dobereiner (1967) obtained results indicating response to molybdenum in dry beans; however excess of this element did more harm to the symbiosis than to plants growing with combined nitrogen. Recent results from our laboratory have shown large increases in forage legume yields due to micronutrient fertilization, once soil acidity and major element deficiencies have been eliminated. Using dry beans (Cv. Venezuela 350) and a Red Yellow Podzolic Soil (ultisol-Udult), with pH = 5.1 but without aluminum and manganese toxicity, a great increase in acetylene-reducing activity and total plant nitrogen was obtained with liming and Mo fertilization. The application of Mo without liming showed no effect (Fig 4). The results of grain yield in this experiment are shown in Table 4. With 1 ton/na lime the increase due to molybdenum fertilization was 220 percent. Similar experiments were carried out using 6 dry bean varieties and 6 different soils, including 3 soils from cerrado. Only one bean variety did not get any benefit from molybdenum fertilization: the greatest responses were obtained with cvs. Venezuela 350 and Rico 23. All six soils tested showed response to molybdenum, once pH was raised to 5.8 - 6.0. However the plants on the soils from cerrado showed poor growth, with less increase due to molybdenum. The results of these experiments and from experiments still in progress indicate that dry bean shows specific requirements for Mo uptake from the soil. These results agree with those obtained by Siqueira (1976) with 4 soils from cerrado in which Mo fixation in the soil was almost nil above pH 5.8, independently of the capacity of the soil to fix Mo at a lower pH (Fig. 5).

Peres, Nery and Franco (1975) showed a generalised Mo deficiency in soils from Rio de Janeiro State and Baraglia (1975), confirmed by Franco, *et al.*, (results not published) from São Paulo State.

I would like to mention here the effect of zinc, even though it does not have a specific role in the *Rhizobium*-legume symbiosis, because the cerrado soils and several others tropical soils show generalised deficiencies of this element. (Jain, 1972; De-Polli, 1975; North Carolina Univ. Ann. Report, 1974).

CONCLUDING REMARKS

The present low yield of grain legumes in the tropical areas is not being limited by potential of the symbioses to fix nitrogen.

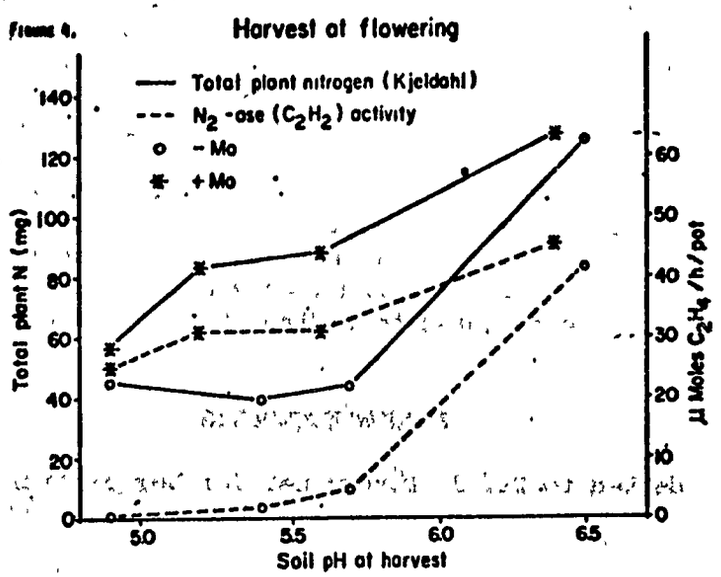

Figure 4. Nitrogenase and total plant nitrogen of *Phaseolus vulgaris* as affected by liming and molybdenum fertilization. Each point is a mean of 4 blocks.

Table 4. Effect of lime and molybdenum fertilization on *P. vulgaris* grain yield in a red yellow podzolic soil[a].

lime	pH at	Mo (kg/ha) as molybdic acid 0		0.25		0.50		2.00	
m.t./ha	harv.	g/pot	% inc.[b]	g/pot	% inc.[b]	g/pot	% inc.[b]	g/pot	% inc.[b]
0	4.9	4.1	-	7.0	71	9.2	124	6.4	56
1	5.3	7.6	85	22.5	448	24.5	494	19.6	376
2	5.6	14.2	246	31.1	657	31.0	653	29.7	623
4	6.4	37.3	807	39.6	864	41.6	913	40.5	885

a Plants growing in pots containing 9 kg of soil. Each value is a mean of 4 pots.

b Increase in relation to yield (underlined) of treatment without lime but with basic fertilization PK and minor elements (except Mo).

The barriers to yield increase are the lack of sufficient water throughout the growth of the crop; the absence of proper *Rhizobium* strains; and the inadequacy of plant nutrients.

Phosphorus deficiency and soil acidity plus aluminum and manganese toxicity are recognized as limiting factors. However these and potassium

deficiency may be diagnosed by soil analysis and corrected. Special attention must be given to situations where phosphorus fixation in the soil occurs. This is an area where much has to be done.

After the major limiting factors have been corrected, to increase production one needs to look to minor element deficiencies, especially molybdenum in acid soils. Differences between plant species or cultivars have to be defined.

It is doubtful whether fertilization with small amounts of nitrogen will increase symbiotic nitrogen fixation and yield for short cycle grain legumes. Soybean does not require nitrogen fertilization up to three times to increase world mean yields. However a more basic approach has to be taken to define this point better.

ACKNOWLEDGEMENTS

I am indebted to Dr. J. Döbereiner for her criticism of this manuscript.

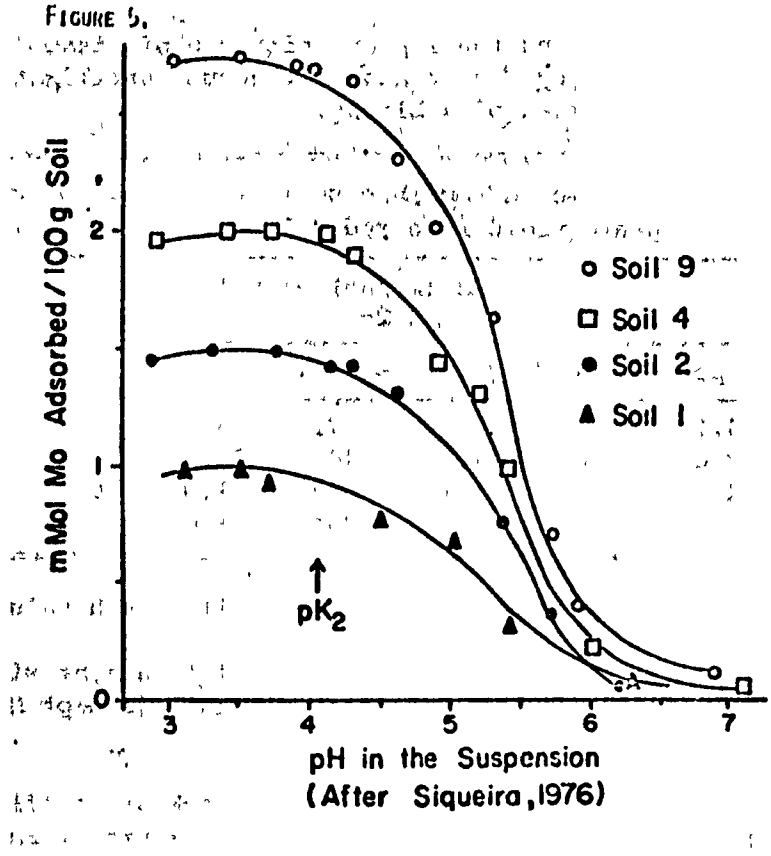

Figure 5. The effect of pH on mllybdenum adsorption in 4 soils from Cerrado. (Siqueira 1976).

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NUTRITIONAL RESTRAINTS ON LEGUME-SYMBIOSIS

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ABSTRACT

The paper discusses the prime effects of plant nutrients (other than acid soil factors) on legume-symbiosis, in relation to tropical and sub-tropical pasture systems. The nutrients have been discussed in three groups, firstly those that have a direct effect on initiation and development of nodules, secondly those that influence the efficiency of the symbiosis, and thirdly those that are concerned directly with plant metabolism and growth independantly of the symbiosis.

Each nutrient is discussed in relation to initiation and development of nodules, the efficiency of the legume-*Rhizobium* symbiosis and the overall effect on quantitative aspects of plant dry matter and nitrogen production. Species comparative results are indicated and attempts made to explain why species differ in their response to a given nutrient. Attention is directed to critical nutrient concentration, nutrient uptake and translocation, and root morphology.

Quantitative aspects of nutrient sufficiency, interaction of nutrients, competition between species in a legume-grass system, and of pasture management are discussed.

INTRODUCTION

The cultivation of leguminous plants is probably the most important method of adding nitrogen to the soil-plant system and furthermore much of the inheritance of soil N may have resulted from legume in the past (Henzell, 1962).

Nutritional factors may affect the production of N by legumes in three ways. They may act directly on the initiation and development of nodules, they may influence the efficiency of the legume-*Rhizobium* symbiosis, or they may play an essential role in plant metabolism and growth independant of the symbiosis. The plant nutrients do not necessarily fall into three simple categories because in some instances a nutrient may have multiple functions e.g. molybdenum is not only essential in relation to the second group of factors but also to the third group; similarly phosphorus acts in all three groups. Furthermore the success of nutrients in the first and second groups is dependant on an optimum

supply and efficiency of all the other nutrients and vice-versa. Factors that are important in relation to initiation and development of nodules are calcium, certain acid soil factors, boron and to lesser degree phosphorus and sulphur. The principal effects of soil acidity may be resolved into hydrogen ion concentration, deficiencies of calcium and molybdenum (not 'initiation'), and excessive amounts of aluminum and manganese (Hewitt, 1952; Schmehl *et al.*,1950), see second paper, Session 3 of these proceedings. The main nutrients to directly affect the efficiency of symbiosis are molybdenum, phosphorus, sulphur, calcium and boron. The effect of nutritional factors on root development and their functioning should be stressed. Certain nutritional factors have adverse effects on root morphology, e.g. Ca and B deficiencies, H and Al excess, and poor aeration. In addition most nutrients affect the root weight ratio of plants.

This paper discusses each nutrient in relation to initiation and development of nodules, the efficiency of the legume-*Rhizobium* symbiosis, and the overall effect on plant growth with emphasis on tropical legumes. In addition aspects of nutrient interaction and quantitative requirements will be discussed.

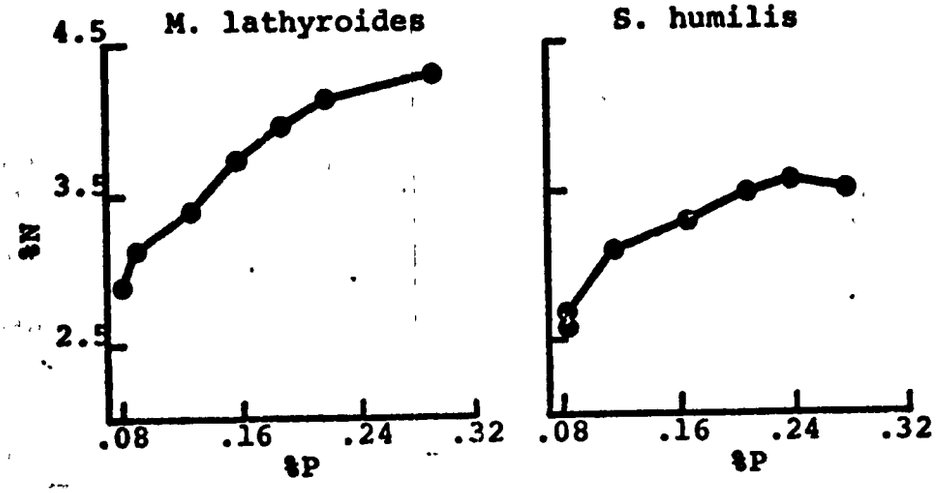
PHOSPHORUS

The gross effect of phosphorus on legume and nitrogen production is contained in the effect on plant dry matter production and on the nitrogen concentration of the plants. Commensurate with addition of phosphate to a soil-legume system there is usually an increase in both phosphorus and nitrogen concentration in the plant. Figure 1. shows the relationship between phosphorus and nitrogen concentration in the tops of two tropical pasture legumes.

Information on a range of tropical legumes is available (Andrew and Robins 1969b). Similar relationships have been shown for a number of pasture and crop legumes (Singh, 1958, Norman, 1959; Parsons and Davies, 1960; Mclachlan and Norman, 1961; Gates, 1974).

The increase in legume nitrogen concentration due to phosphorus addition may be compounded of such factors as a) time of nodulation, b) extent of nodulation, c) duration of symbiosis, and d) general efficiency of symbiosis. Little information on tropical pasture legumes in relation to the above factors exists except for *Stylosanthes humilis*. Gates (1974) studied the response of *S. humilis* to added P in soil pot culture and found that P had a beneficial effect on the initiation of nodules; they were first detected at day 11 in high P plants, but not until day 14 in low P plants. Furthermore he showed that nodule numbers, volumes, and dry weights were enhanced by P supply. Nodule relative growth rates were stimulated from 0.3 g/g/day at low P treatment to 0.7 g/g/day at high P treatment over days 23-26. It was also observed that nodules became pink earlier at the high P treatment than at the low. The assimilation of nitrogen by the whole plant was increased from 17 mg/g nodule dry weight/day at low P treatment to 53

FIGURE 1. EFFECT OF P ON THE CONCENTRATIONS OF N AND P IN THE TOPS OF TWO TROPICAL PASTURE LEGUMES.



mg/g/day at high P supply over days 23-26. In contrast to this, Souto and Dobereiner (1969) found that while P addition increased dry matter, nodule growth, and total nitrogen of *Glycine wightii*, there was no effect of treatment on total nodule weight or N fixed per unit weight of nodule. Results of Eira *et al.*, (1972) differ between species, e.g. P treatment increased the N concentration, dry matter and total N and nodulation in *Macroptilium atropurpureum* but decreased the N concentration in *S. gracilis* and *G. wightii*.

In the above experiments soil pot culture was used in each case. This poses the question as to whether the enhanced effect of P addition was a prime effect on nodule initiation and weight or whether the apparent superior nodulation resulted from improved nutrition and photosynthesis of the host plant following P addition. Furthermore P treatment may result in more efficient extraction of soil N. However the overall relationship of N and P concentrations in plant tops in relation to D.M. yield suggests that there is an unequivocal effect of P on N production by legumes e.g. the concentration of P in the plant coinciding with maximum D.M. production is usually less than that corresponding to maximum N concentration. In addition there is an upper N concentration limit for each species and this may vary between species (Figure 1) e.g. that for *S. humilis* is less than that for *M. lathyroides*. The same relationships, N to P concentrations, may be used to estimate the relative efficiencies of the legume-*Rhizobium* symbioses in relation to P, e.g. the slopes of the lines over the full treatment range for *S. humilis* is less than those for the other legume (Figure 1).

The quantitative aspects of total D.M. and of N production as affected by P treatment is of course dominated by the D.M. component. In this regard it is well recognised that plant species differ: within the tropical legume group *S. humilis* and *L. bainesii* are very efficient

species (Andrew and Robins 1969a). The response of *S. gracilis* to added P has been found to be less than that of *Centrosema pubescens*, *G. wightii* and *M. atropurpureum* (Jones and Freitas, 1970). Differences also occur within genera and species, e.g. *Stylosanthes* (Jones, 1974). The relative responses of legumes to applied P are controlled in part by seed size, relative growth rate, perenniality, root patterns, symbioses, internal P requirements and utilization, and form of applied phosphate and soil conditions. Legumes that have a relatively small response to added P usually have relatively low critical percentages of P and furthermore they have a capacity for short and long term utilization of certain insoluble phosphates such as rock phosphate. The explanation for the latter may lie in their soil-plant interactions or in biological symbioses (Mycorrhiza) or both (see Session e(5)). Phosphorus critical percentages for a range of tropical pasture legumes has been given by Andrew and Robins (1969a).

Differences in response of legumes to added P may be attributed to many factors (Andrew and Jchansen, 1976). Two of these are discussed here. (a) Critical P concentration:- Critical concentrations of P in the tissue may be used to estimate the P internal requirement. *S. humilis* and *L. bainesii*, species reputed to be efficient phosphate users, had P critical concentrations of 0.17% whereas *D. uncinatum*, a very responsive species, had a value of 0.23%. In the above investigation by Andrew and Robins (1969a) the range of concentrations was 0.17 to 0.25%, however the principle outlined above is not in agreement with all data, e.g. *M. sativa* is considered to be efficient, especially in relation to rock phosphate usage, but has a relatively high P critical concentration. Furthermore, other factors, plant and soil, may dominate the degree of relative response, e.g. a deep rooting species such as *M. atropurpureum* grown in a soil with reserves of soil P at depth has definite advantages over shallow rooting species. (b) Uptake mechanisms:- Rate of P uptake experiments of using excised roots (Nissen, 1973) and the application of kinetics to the data (Hagen and Hopkins, 1955) have assisted in the explanation of differences between species. Similar techniques were applied to a group of four tropical legumes and barley (Andrew, 1966). In that experiment the P uptake rate per gram root by *S. humilis* was considerably greater than that of *M. lathyroides*, *D. uncinatum*, *M. sativa* and *Hordeum vulgare* at low substrate concentration ($1x10^{-6}$M P). Kinetic interpretation of those and other data showed that two first order reactions were concerned in the uptake phase and that these differed in magnitude between the species. Secondly Km's also varied between the species. In another experiment concerned with an Al study (Andrew and Vanden Berg, 1973), the uptake of P per gram root and translocation of P from root to top by intact plants of *S. humilis* and *L. bainesii* exceeded that of *D. uncinatum* and *M. lathyroides*, ($1-10^{-5}$M P substrate).

Polyvalent cations, especially Ca, stimulate P uptake by plants (Pettersson, 1975); optimum substrate Ca concentration is approximately $2x10^{-4}$M Ca. Some workers have suggested that plants with high internal Ca concentrations have a propensity for P uptake and utilization, however the author's experience does not fit this hypothesis, e.g. the Ca concentration in *S. humilis* is always relatively high (grown on all substrates)

while that of *L. bainesii* is low, and yet both species are efficient with respect to P usage (Andrew and Robins, 1969a; Andrew and Johnson, 1976). For example the Ca concentrations in the tops of *S. humilis* and *L. bainesii* grown in a 0.125 mM Ca substrate were 1.32 and 0.38% respectively.

Many other factors are important in P usage especially symbioses such as Mycorrhiza (Mosse *et al.*, 1976), see Session 3(5).

SULPHUR

The essentiality of sulphur as a plant nutrient has been known for many decades, and early investigators were able to relate some of the functions of sulphur to plant protein production. For a treatise on the biochemistry of symbiotic nitrogen fixation in legumes see Bergersen (1971). Published data on the responses of tropical pasture legumes to added sulphur are very few and indeed it is only in the last two decades that attention has been given to the temperate pasture legume situation. However increased knowledge of sulphur responsive areas in northern Australia has resulted in attention being given to sulphur diagnosis (Andrew *et al.*,1974; Jones *et al.*,1975).

In situations of responses to added sulphur or S compounds, both legume D.M. and concentrations of plant S and N are increased. The author is not aware that plants fail to nodulate under conditions of acute deficiency, however there is considerable evidence that the addition of S compounds to a S deficient soil will increase the nodulation of tropical legumes (Andrew *et al.*,1952, Gates *et al.*,1971; Gates, 1974; Andrew, in preparation; Robinson and Jones, 1972). Gates (1974) studied that effect of added S on *S. humilis* in soil culture and found that the addition of S did not affect the time of initiation of nodulation, but total nodule dry weight per plant was increased. Furthermore the relative growth rate of nodules was not increased by S addition. However the evidence of a close relationship between N and S concentrations in tropical legumes is unequivocal (Robinson and Jones, 1972; Andrew, in preparation), Figure 2.

In Figure 2 correlations between N and S concentrations are shown. There is a suggestion of a plateau on the S axis. This is similar in principle to that formed in the P-N relationship (Figure 1). There is also evidence that the effect of S on N concentration extends beyond the level of S corresponding to maximum D.M. of tops. This raises the ques question as to whether one should fertilise a pasture for maximum D.M. or for maximum N concentration and hence maximum N production, however economics will govern that decision. Based on laboratory experimentation results so far, it is difficult to establish differences between legumes in their response to added S. However, certain field experiments indicate that shallow rooted species are at a disadvantage and usually respond more than deep rooted species, e.g. *Medicago denticulata* is more responsive than *M. sativa*, similarly *D. intortum* is more responsive than *M. atropurpureum*. Furthermore soil adsorbed sulphate tends to accumulate

FIGURE 2. EFFECT OF S ON THE CONCENTRATION OF N AND S IN THE TOPS OF TWO TROPICAL PASTURE LEGUMES.

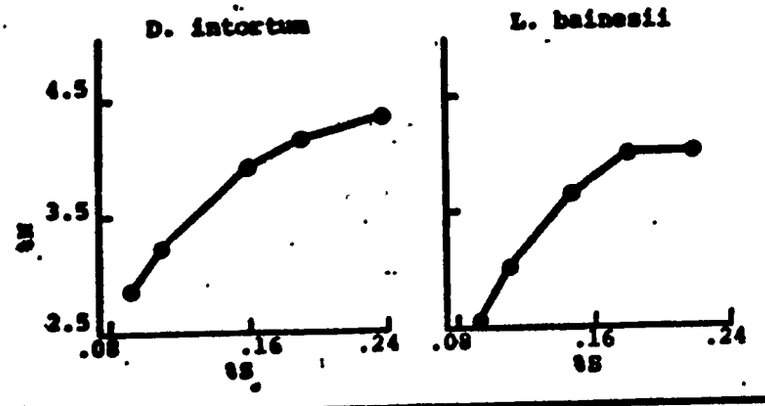


FIGURE 3. EFFECT OF MO ON THE D.M. YIELD AND N CONCENTRATION IN THE TOPS OF D. INTORTUM.

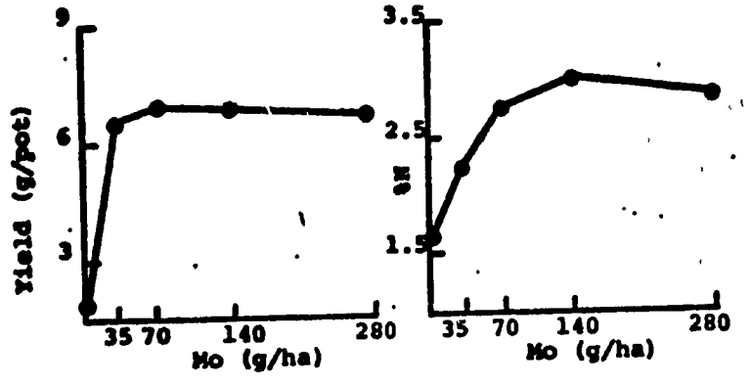
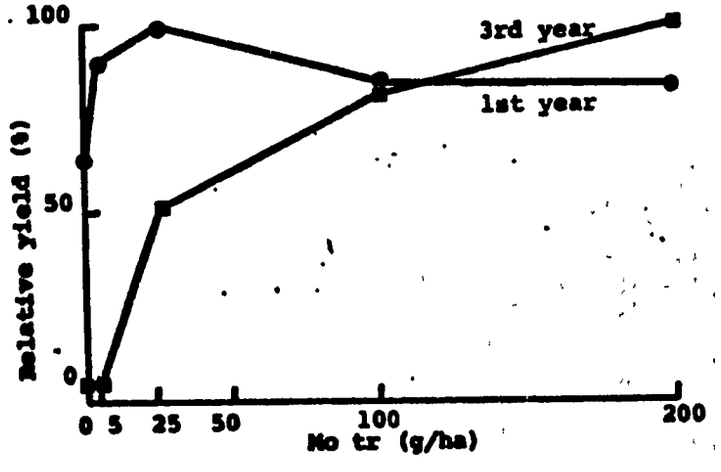


FIGURE 4. THE EFFECT OF INITIAL APPLICATIONS OF MOLYBDENUM ON THE RELATIVE YIELD OF D. INTORTUM OVER A THREE YEAR PERIOD.



in the "B" horizon of soil, commensurate with an increase in clay content especially in medium to high rainfall areas e.g. in a typical Krasnozem soil in Northern Australia the phosphate-extractable S increased from 18 ppm in the surface soil (0-10 cm) to 520 ppm in the 150-180 cm layer (Probert, 1974). Subsequently Probert and Jones (unpublished data) have shown that this method of diagnosing soil S status in tropical Australia has merit. Research has also been accomplished in the use of plant S for diagnosing the S status of tropical and sub-tropical legumes (Andrew *et al.*,1974). Sulphur critical percentages for seven tropical legumes range from 0.14% to 0.17%, and for five temperate legumes from 0.17% to 0.20%. Further data are available for *S. humilis* (Jones *et al.*, 1971). However Robinson and Jones (1972) have shown that S is readily transported in *S. humilis;* a large proportion of the total plant S being in the seed.

MOLYBDENUM

Several reviews have been concerned with role of Mo in the nutrition of legumes and non-legumes (Anderson, 1956; Bergersen, 1971). Early experiments with pasture legumes, primarily in temperate areas, indicated a close relationship between D.M. production and nitrogen concentration (Anderson and Spencer, 1950; Anderson, 1956). Subsequently, numerous reports have been published of tropical legumes responding to Mo addition (Truong *et al.*,1967; t Mannetje *et al.*,1963; Kerridge *et al.*, 1972; Franca *et al.*,1973; Eira *et al.*,1972; Ruschel *et al.*,1966). Figure 3 indicates the effect of added Mo on the D.M. and N concentration of *D. intortum* grown on a krasnozem soil.

There are no records of Mo deficiency preventing nodulation of legumes, indeed the reverse is usually the case. Detailed investigation with *T. subterraneum* plants have shown that Mo deficient plants have more nodules than do normal plants (Anderson and Thomas, 1946; Anderson and Spencer, 1950). The latter authors also showed that the numbers of nodules could be decreased by applying Mo or combined nitrogen, and that the effect was attributable to N deficiency, brought about by Mo deficiency and the resultant increase in susceptibility of the plants to infection with *Rhizobium*. Similar results have been achieved with tropical pasture legumes ('t Mannetje *et al.*, 1963; Kerridge *et al.*,1972; Johansen and Kerridge, personal communication). In all quoted cases the nodules on Mo deficient plants were numerous, small in size, and internal nodule tissue was white, pale pink, or greenish in colour: by contrast, the nodules of Mo sufficient plants were large and pink in colour (red haemoglobin pigment). Nodules with strong pigment colour have been shown to be most active in N fixation (Virtanen and Laine 1946). In those cases where Mo addition has reduced nodule numbers but increased their size the amount of N fixed per unit weight of nodule has also increased (Anderson and Spencer, 1950; Ruschel *et al.*,1966).

Crop plants differ in their response to Mo, e.g. in an early publication (Johnson *et al.*,1952) legumes and graminae were shown to be non

responsive groups whereas *Chenopodiaceae, Compositae, Cruciferae, Solanaceae* and *Cucurbitaceae* were responsive groups. However further research has shown that the degree with which a legume will respond depends on the available nitrogen in the substrate. This aspect is extremely important in pasture development and maintenance. In a newly established pasture, especially if established in well fallowed land, available soil N could well be sufficiently high to allow optimum growth of the legume and thus not have a response to added Mo. The relative Mo requirement of a legume for enzymatic transformation of nitrate to ammonium forms is less than that for the efficient functioning of the symbiosis in the nodule. It is for this reason that Mo deficiency in pasture legumes is primarily reflected as N deficiency, although specific effects of Mo deficiency on metabolism of N fed legumes result in visual symptoms specific to Mo, (for symptoms on *D. intortum* and *M. atropurpureum* see Andrew and Pieters 1972a, 1972b). Mixed pastures inevitably become acutely N deficient unless the legume is actively fixing N and it is under conditions of depleting available soil N that legume responses to added Mo are expected. This implies that field experiments are more accurate than pot experiments or soil chemistry for the determination of Mo deficiency, and furthermore it is advisable to continue field experiments for at least three years or more. Figure 4 shows the time effect of Mo response on *D. intortum* grown on a podsolic soil; this effect may also be compounded by soil fixation of Mo in time.

Within the pasture legumes the *Medicago* species have been shown to be more responsive than the *Trifolium* species (Hewitt 1958). Differences occur between the tropical legumes: Johansen and Kerridge (in preparation) have shown that the relative order of responses of a group of tropical legumes was, *G. wightii* > *D. intortum* > *M. atropurpureum* > *M. sativa* > *L. bainesii* ~ *S. guyanensis*. Furthermore the longevity of Mo effectiveness when added to soils of varying types compounds the degree of response for each legume.

BORON

The role and physiological action of B in plants have been reviewed (Gauch and Duggar 1954; Whittington 1956), however little attention was given to specific roles of B in legume nutrition, but at that time it was established that B was in part concerned with protein metabolism because of the accumulation of amino-acids and amonium ions and the decrease of protein in B deficient alfalfa plants (Scripture and McHargue 1943). Boron is not considered essential for rhizobia but it is required for a normal development of roots and nodules (Mulder 1948; Warington 1926). However B is reported to be beneficial for the fixation of N by *Azotobacter* (Jordan and Aderson 1950). In the work of Brechley and Thornton (1925) the N concentration in the tops of *Vicia faba* was increased from 2.46 to 3.43% by B addition to the substrate. The early investigation of Mulder (1948) on the garden pea showed that plants grown in water culture in the absence of added B did not nodulate and at very low levels of added B the nodules were very small, light yellow colour, somewhat slimy and apparently were unable to fix N. Mulder

postulated that a small amount of B allowed some nodules to develop and once formed they fixed N normally and independently of the occurrence of pronounced symptoms of B deficiency in the tops. From that experiment he suggested that the requirement by the host plant for B was greater than that of the nodule symbiosis. However in subsequent experiments (soil culture Mulder (1948) showed that B deficiency gave poor growth, and leaves showed a light green and later a yellow colour indicative of N deficiency; nodules were present but were black and were shrivelled at harvest.

Very few data are available for tropical pasture legumes. The results of Loustalot and Telford (1948) with kudzu have shown an effect of lack of B in reducing the size of roots and the number and size of nodules. Franca *et al.*,(1973) showed that added B increased the D.M., total N, and nodule weight of *G. wightii* and decreased the concentration of N, P, K, Ca and Mg in the plant. In this instance there was presumably no direct effect of B on the symbiosis. Eira *et al.*, (1972) ascertained the effect of a micro-nutrient mixture (B, Mo, Fe, Cu, Zn) on three legumes. Their results showed that the treatment increased D.M., nodule numbers, nodule weight and N% and total N in *M. atropurpureum;* and increased D.M. and percentage and total N in *S. gracilis*. However the effects observed may not have been attributable to B. In an allied crop legume *P. vulgaris*, Ruschel *et al.*,(1966) showed that added B increased plant D.M., nodulation, and N% (from 2.80 to 3.93%N). The combined effect of added B and $MgSO_4$ increased the N% to 4.6. These authors considered that more B was necessary for optimum nodulation than for D.M. production.

The effect of B deficiency on growth, including root morphology, of some tropical legumes has been established under laboratory water culture conditions *(D. intortum* - Andrew and Pieters 1972a; *M. atropurpureum* - Andrew and Pieters 1972b). No prime cases of B deficiency in the field have been observed by the author for tropical legumes. Responses that have been obtained experimentally have been as a result of liming, however it is now recognised that under Australian tropical conditions there is currently no need to lime for the legumes in use. As a result of these findings no species comparative investigations have been made in Australia, and the author is not aware of recorded results elsewhere.

MAGNESIUM

The essentiality of Mg as a plant nutrient has been known since about 1860. Meyer and Anderson (1939) reviewed the Mg investigations up to that time. Subsequently the vast majority of the investigations into Mg status of plants and its diagnosis has involved horticultural, floricultural and plantation crops. In the excellent compilation by Chapman (1966) of results of Mg studies, only one or two investigations out of approximately 240 quoted were concerned with pastures; none of these were concerned with tropical pastures. Subsequently considerable attention has been given to Mg in relation to grass tetany "hypomagnesemia", particularly in Europe and North America. This problem is not necessarily

directly related to Mg deficiency but rather a cation-anion inbalance. However even under those conditions the author does not know of a simple Mg deficiency in the legume. It would appear that in horticultural, floricultural, plantation pursuits, and in pasture (grass tetany) Mg deficiency, an imbalance is almost invariably induced by relatively high use of ammonium and K compounds and liming. The author is not aware of a direct response or even a useful induced response to Mg for tropical pastures grown in northern Australia.

However this present state of knowledge should not allow complacency to develop with respect to Mg in pasture systems. It is necessary to understand its relationship and functions in pasture species. Norris (1958, 1959) pointed out that *Rhizobium* needs Mg. He indicated that Ca was an essential nutrient but could be considered a micro-nutrient for that organism, and drew attention to the relative requirements of Mg and Ca in contrast to the generally accepted hypotheses of liming for legume at that time. He also drew attention to several publications concerning Mg compounds, e.g. Graham (1938) obtained greatly increased numbers of soybean nodules (in the presence of adequate Ca) as the amount of Mg in the clay colloid increased. Norris suggested that the enhancement may have been due to an increase in the numbers of bacteria available for nodulation. He also cited the work of Anderson and Spencer (1948) and Spencer (1950) in similar context.

Following on from the work of Norris several researchers have investigated the role of Mg in several tropical pasture legumes. Franca *et al.*,(1973) did not obtain any effect of Mg on D.M. or N% in *G. wightii* grown in a "cerrado" soil. A positive response to Mg was obtained by Ruschel *et al.*,(1966) using *P. vulgaris* e.g. in the presence of added Mg and B the N% in plant tops was increased from 3.93 to 4.60%. However in this instance Mg was used in the form of Mg SO_4, therefore the response may have been due to S and not Mg.

There are also reports in which the application of $MgCO_3$ and dolomitic materials have produced superior results to $CaCO_3$. Interpretation of such results requires careful and detailed assessment, especially soil chemical measurements and multi-element analysis of plants.

The author has investigated the role of a wide range of Mg treatments (8) on the growth of six tropical pasture legumes in sand culture without added combined N.

Figure 5 shows the results for D.M. and the Mg : N relationship in tops of two tropical legumes *(M. atropurpureum* and *C. pubescens)*. The principal features of these results are, a) both species were drastically reduced in D.M. by a deficiency of Mg and both species displayed typical Mg foliar deficiency symptoms, b) *M. atropurpureum,* especially, was adversely affected by high substrate concentrations of Mg, c) Mg concentrations in the top of *M. atropurpureum* grown at high Mg substrate concentrations were greater than those for *C. pubescens* at comparable treatments, d) Mg deficiency reduced the concentration of N in the tops of both species, e) Mg toxicity also reduced N% in *M. atropurpureum.* The relationship between Mg and N concentrations in the two species are shown in Figure 5. Nodules existed on plants at all treatments. Details of results for these two species and others, including full

FIGURE 5. EFFECT OF Mg ON THE CONCENTRATIONS OF N AND Mg IN THE TOPS OF TWO TROPICAL PASTURE LEGUMES.

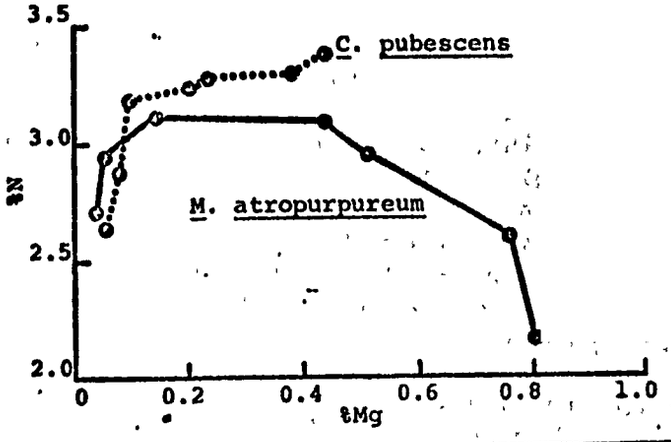


FIGURE 6. EFFECT OF Zn ON THE D.M. YIELD OF TWO PASTURE LEGUMES.

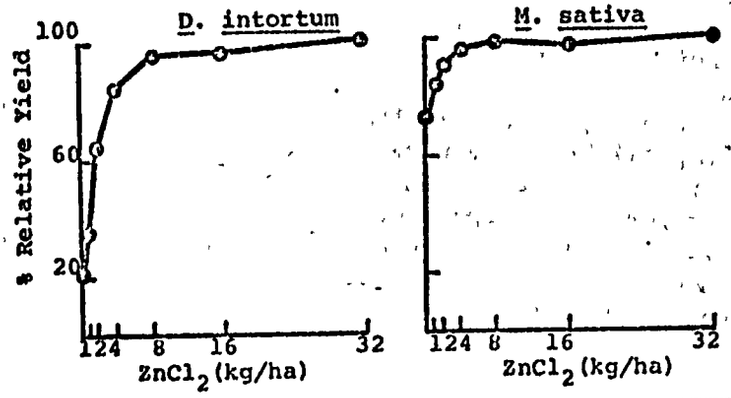
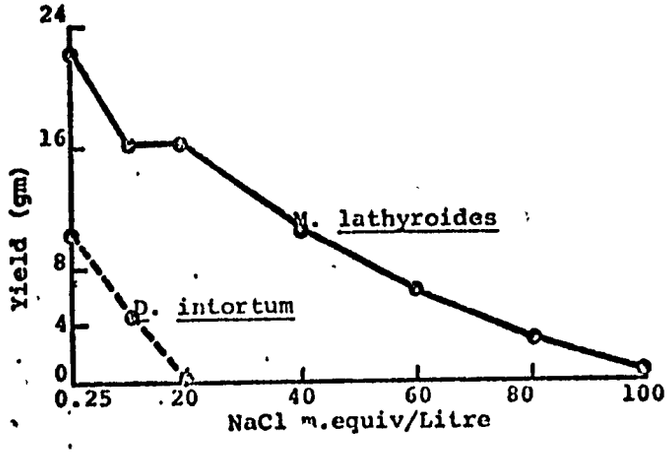


FIGURE 7. EFFECT OF NaCl ON THE D.M. YIELD OF TWO TROPICAL PASTURE LEGUMES.



chemical composition are being collated for publication (Andrew, in preparation).

ZINC

Many of the early published results relating to Zn deficiency pertain to horticultural crops (Chapman 1966). Subsequently it was recognised that many of the agronomic crops also responded to additions of Zn on certain soils, including corn, temperate cereals, and field bean (Viets *et al.*, 1954). Numerous field experiments in California (Hoagland *et al.*, 1936) and in Australia (Riceman 1945, 1948) produced results showing that *M. sativa* was generally a non-responsive species compared to other leguminous and non-leguminous species. Riceman also compared the response of *M. sativa* and *T. subterraneum* to Zn addition in water culture. His results showed a greater response by *T. subterraneum* than *M. sativa;* in both species roots were affected to a lesser degree than the tops. Millikan (1953) produced similar results. In contrast to these data, those of Carroll and Loneragan (1968), obtained by growing species in a flowing nutrient culture system, showed that M. sativa was more sensitive than *T. subterraneum* to low levels of Zn supply; however these authors acknowledged that this result was in contrast to those of field experimentation. The data of Riceman (1953) and of Carroll and Loneragan (1968) were obtained in a culture system having ample combined N supplied. Dry matter responses in some tropical pasture legumes have been recorded (Franca *et al.*, 1973; Andrew and Bryan 1955). Results from a species comparative study (Andrew, unpublished) cover seven tropical pasture legumes and *M. sativa* grown in a heavy textured soil (pot culture). Figure 6 shows the D.M. responses for two species.

The relatively small response of *M. sativa* is in agreement with data of Hoagland *et al.*, (1936) and Riceman (1945, 1948).

Data concerned with the effect of added Zn on N concentration in pasture legumes is very meagre. The report of Franca *et al.*, (1973) indicates that Zn increased the D.M., nodule weight, and N production of *G. wightii;* however these results did not show any effect on N%. In the work of Andrew (unpublished) above, no increase in plant N% was observed, indeed the reverse occurred i.e. a reduction. This apparent reduction is explained by the relatively low D.M. at nil or small Zn soil additions and commensurately high levels of soil N available. Results from further experimentation by Andrew (in preparation) involving the growth of *D. intortum, G. wightii* and *M. atropurpureum* in water culture but in the absence of combined N show that Zn had an effect on nodulation. In *G. wightii* the D.M. yield of nodules as a percentage of D.M. of roots + nodules increased from 1.0 at the nil Zn treatment to a maximum of 19.7 at the 0.05 ppm Zn treatment. However despite this effect there was no significant effect on the N% in the plant tops.

COPPER

Early investigations were summerised by Arnon (1960). Since that time there have been numerous reports of temperate and tropical pasture species responding to added Cu, however very few comparative studies have been made.

Millikan (1953) compared *M. sativa* and *T. subterraneum* and showed that the response to added Cu was greater in the former species than in the latter, and that the coefficient of utilization of Cu was highest in *M. sativa*. Five tropical and five temperate pasture legumes were compared by Andrew and Thorne (1962) in soil and in water culture. Marked differences in D.M. responses were obtained; *D. uncinatum* was the least responsive and *S. guyanensis* was the most responsive among the tropical legumes. Subsequent work by Andrew showed that *L. bainesii* was a relatively insensitive species. In the soil experiment, plant N concentrations were not affected by Cu additions; in the water culture experiment they were slightly reduced.

Van Schreven (1936) postulated that Cu deficiency must lead to a high level of soluble nitrogenous compounds and poor protein synthesis. His postulation was based on factors that little haemoglobin is formed in nodules in the absence of added Cu (Virtanen 1947), chlorophyll is reduced (Erkama 1947), and carbohydrate metabolism is disturbed (van Schreven 1936). Hallsworth (1958) and his colleagues studied the role of Cu in respect to nodulation and growth of *T. subterraneum*. Their results showed that an insufficiency of Cu caused poor root growth and small nodules principally on the secondary roots whereas in the presence of adequate Cu the root growth was vigorous with nodules developed in dense clusters close to the main root. Furthermore it was shown that the high Cu treatment (0.064 ppm) increased the N% in the plant tops from 2.73 to 3.15%.

POTASSIUM

Potassium is an important nutrient in relation to the long term maintenance of pastures, especially legume based pastures, particularly in medium to high rainfall areas. In some areas of light textured soils and high rainfall K may be necessary at establishment. However published data do not show any K effect on N concentration in legume tops (Albrecht *et al.*, 1948; Anderson and Spencer 1948; Lynch and Sears 1951; Andrew and Robins 1969b). The latter authors worked with tropical pasture legumes: their data showed a reduction in N% resulting from K addition, however these results were obtained from a pot culture study therefore the relatively high N% in the plants of the no-K treatment may have been due to relatively high available nitrate commensurate with poor plant growth. In field situations, legumes plus grasses, the N concentration of legumes responding to soil K additions was not increased (Andrew and Shaw (personal communication); Hall (1970), although the legume N per unit area of land was considerably increased

(see later discussion).

In the publication by Andrew and Robins (1969c) the D.M. responses and K critical percentages for eight tropical and four temperate pasture legumes were given. For other data on *S. humilis* see Gates *et al.*, (1966), and on *C. pubescens* Hamilton and Pillay (1941).

MANGANESE, IRON AND COBALT

It is not possible to deal with these in detail in this paper, however, various reports indicate the significance of these nutrients in plant growth and N metabolism. Most cases of D.M. response to Fe and Mn have occurred in horticultural and agronomic crops. The author is not aware of deficiencies of these two nutrients occurring in tropical pastures under field conditions. There have been a few examples of induced responses in pot culture resulting from lime and dolomite application, however the use of lime for most species in use in northern Australia is not justified particularly if the user takes advantage of Mo addition in those areas having a Mo x lime response interaction.

It is tempting to suggest that Fe may be important in N fixation by the nodule because of its association in leg-haemoglobin (Virtanen 1956). Research into the role of Mn has been largely concerned with chlorophyll production, carbon assimilation and balance of carbohydrates and their effects on protein synthesis, however the author is not aware of data relating to nodulation or efficiency of symbiosis.

Cobalt has been implicated in legume nutrition especially in relation to *T. subterraneum* (Ozanne *et al.*, 1963 in Western Australia, and Powrie 1960 in South Australia). The responses obtained were on soils of very sandy type and were not consistent or of long duration. There are several known areas of Co deficiency in animals in which no plant response in D.M. or N% have been obtained (e.g. North eastern portion of Cape York, Queensland, Australia (Winter - personal communication)).

SALT (NaCl)

Problems of pasture establishment on saline soils have been encountered both in irrigated and non-irrigated situations (Cope 1958; Evans 1967). One of the principal means of mitigating the problem is to use relatively tolerant species, e.g. *T. alexandrinum, M. sativa*. In the sub-tropical areas of Australia, brigalow (a leguminous tree) is well adapted to soils of high salt content (Isbell 1962). The relative tolerances of a group of 16 tropical legumes has been investigated by Hutton (1971) using a sand culture technique. All legumes were reduced in D.M. by the salt treatment (60 mM NaCl). The relative order of tolerance was *M. sativa* > *Macroptilium* > *Centrosema* > *Lotononis* > *Vigna* > *Stylosanthes* > *Glycine* > *Desmodium*. Salt treatment did not prevent nodulation, but decreased the N% of the tops 2 to 15%. Gates *et al.*, (1970) studied the effects of salt on a range of *G. wightii* cultivars. Their results showed that the cultivars could be

divided into groups according to their tolerance to salt. The most tolerant group was more efficient at excluding sodium uptake and to a lesser degree chlorine uptake, than the very susceptible groups. A more detailed study on *G. wightii* with respect to nodulation by Wilson and Norris (1970) showed that salt treatment reduced the number of nodules per plant and also reduced the amount of N fixed per unit weight of nodule, and renewed nodulation and increased efficiency occurred following the removal of salt stress. These authors indicated that developed nodules proved quite resistant to stress. In a subsequent study on *G. wightii* Wilson (1970) showed that salt treatment reduced the nodule/root weight ratio but had little if any effect on nodule/lamina weight ratio. Nitrogen concentration was lowered in all plant parts but to a lesser degree in the roots than the other portions.

In the experiments of Gates, Wilson and colleagues the salt treatment was imposed on established plants and removed at a later stage in order to study N production efficiency and recovery. Andrew (in preparation) studied the establishment and growth of twelve tropical pasture and forage legumes in a range of 8 salt concentrations (0.25 to 150 m equiv. NaCl/l) maintained. All species were reduced in D.M. production by increasing salt treatment. Differences occurred between species but the tolerance of *M. sativa* surpassed that of all tropical species. Figure 7 shows the relative responses of two species to salt treatment.

In some instances plants grown at high salt treatment failed to nodulate; at intermediate treatment roots were brown at harvest and there was little evidence of new nodule formation. Nitrogen concentrations in the plant tops were reduced by relatively high salt treatment. Observations by Evans (1967) on a group of legumes sown into a salty area of the Queensland coast resulted in the following order of tolerance: *M. sativa* > *L. bainesii* > *M. atropurpureum* > *D. intortum* > *D. uncinatum*. Russell (1976) compared 11 tropical legumes, 10 temperate legumes and 11 tropical grasses in pots of a solodic soil with increasing levels of NaCl. *M. sativa* was the most tolerant legume, *M. lathyroides* and *M. atropurpureum* were almost equivalent to *M. sativa*, whereas the most sensitive legumes were *D. uncinatum* and *T. semipilosum*.

GENERAL

The foregoing discussion has been primarily in relation to the effect of nutrients on nitrogen production based on the nitrogen concentration in plant tops. However in addition, there are several aspects of nutrition that are important in maximising nitrogen production from a legume based pasture.

1. Sufficiency of all nutrients

Data shown in Figure 1, were obtained from rate of application experiments in which the application rates were chosen so as to produce a D.M. yield plateau. From these data critical percentages may be determined (Macy 1936) for the conditions of the experiments, however

Table 1. Effect of Varying Rates of KCl on the D.M. Yield and Chemical Composition of *D. intortum*

Treatment		Yield	Chemical Composition		
KCl (g/pot)	Equiv (kg/ha)	(g/pot)	N (%)	P (%)	K (%)
Nil	Nil	2.1	4.4	0.35	0.31
0.04	25	5.7	3.8	0.18	0.34
0.08	50	6.9	3.3	0.16	0.53
0.12	75	6.8	3.3	0.17	0.74
0.16	100	7.0	3.1	0.15	0.87
0.20	125	6.9	3.3	0.14	1.02
0.24	150	6.8	3.2	0.15	1.22
0.32	200	6.9	3.4	0.15	1.50

Table 2. Effect of P, Lime, S and Mo on the Mean D.M. Yield of *M. atropurpureum* grown in a Podsolic Soil

Treatment	Yield (g/pot)
$P_0Ca_0S_0Mo_0$	0.52
$P_1Ca_0S_0Mo_0$	1.56
$P_1Ca_1S_0Mo_0$	2.37
$P_1Ca_0S_1Mo_0$	1.98
$P_1Ca_1S_1Mo_0$	3.74
$P_1Ca_0S_0Mo_1$	1.61
$P_1Ca_1S_0Mo_1$	4.50
$P_1Ca_0S_1Mo_1$	2.16
$P_1Ca_1S_1Mo_1$	4.57

it may be questioned as to whether the turning points in the curves correspond to nutrient sufficiency. Induced secondary nutrient deficiencies can also be responsible for producing a D.M. yield plateau, e.g. in Table 1 the plateau commenced at the third treatment corresponding to a very low plant K concentration and indeed a low P%, therefore beyond that point the plant D.M. response to added K was limited by a secondary P deficiency.

In this instance the data are not suitable for ascertaining the maximum D.M. potential or for the determination of diagnostic criteria. In practical usage it may be known from experience that no secondary deficiencies exist or as a precaution basal applications of suspected deficiencies may be made or used in factorial combination with the prime nutrient deficiency. In the interpretation phase the sufficiency of nutrients not under test may be judged by foliar analysis and reference to accepted standards (Andrew 1968).

Interaction of nutrients, especially with respect to legume responses, are common. Table 2 is a good example of the interaction of P, S, Mo, and lime on the growth of *N. atropurpureus*.

2. Specific effects on legumes in mixed pastures

Certain nutrients have greater effects on the legume component than the grass component of a pasture. The desired balance of legume to grass will vary with conditions, species and management. However a ratio of 1:2 is highly acceptable. The principal nutrients that usually have a relatively high specific effect on legumes are potassium, molybdenum, sulphur, lime and phosphorus. Potassium and molybdenum examples are discussed here.

It is general experience that the severity of K deficiency in a pasture increases with time and that the grass competes strongly with the legume for available K (Blaser and Brady 1950; Hall 1971). In one situation (Andrew and Shaw, personal communication) the legume component in a *S. humilis* and *Heteropogon contortus* pasture immediately following establishment of the legume was approximately 30% of the total, however after six years K deficiency had become acute and the legume component had been reduced to approximately 20%. In the first season following the addition of K the legume component rose to 57%, based on a 3 month sample period. The amount of N in the above ground legume sample (harvest height 5 cm) in the absence of applied K was 28 kg/ha whereas in the presence of added K the amount was 80 kg/ha. In another field experiment (Johansen and Kerridge, personal communication) the legume content *(D. intortum)* in a pasture that had been established for three years in the absence of added Mo, was 2% of the total pasture whereas in the presence of added Mo the legume component was 40%. Furthermore the N concentration of *D. intortum* in the former case was 1.9% and in the latter, 3.2%. The total N in the legume sample over a 6 month growth cycle was 1.5kg/ha in the absence of added Mo and 40kg/ha in the presence of Mo (cut at 5 cm height).

The specific effect on a legume will be conditioned by the soil available N under some conditions, e.g. if soil N is high the grass component will frequently respond rapidly to the added P and in so doing reduce the legume component and its response. Furthermore the relative responses of individual legumes and grasses must be borne in mind.

3. Diagnosis and management

Attempts to maximise N production by legumes will of course be dependent in part on each essential plant nutrient being at optimum

supply. Diagnosis is therefore extremely important in determining the sufficiency or otherwise of all nutrients. The most efficient method available is the full field factorial experiment; however this method does not lend itself to every day use for all situations. Alternatively, soil and plant chemistry, pot culture experimentation and visual foliar symptoms may be used. In all cases the researcher must be aware of all factors affecting plant growth and the interactions of the various nutrients and other factors. The choice of method or methods will be largely governed by the situation encountered (Andrew 1968).

Nutrition investigation is only one aspect in the establishment and maintenance of an efficient soil-plant-animal production system, e.g. an excessively high intensity of grazing can seriously deplete the majority of pasture legumes (for details see paper by Jones in this workshop).

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THE ROLE OF MYCORRHIZA IN LEGUME NUTRITION ON MARGINAL SOILS

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Yield potential and environmental constraints on the growth of tropical legumes have been discussed in previous papers. In many tropical soils scarcity of available phosphate is an important determining factor because it affects both plant growth, and nodulation. It is well known in practice that an adequate phosphate supply is necessary for satisfactory nodulation and symbiotic nitrogen fixation (van Schreven, 1958; Hallsworth, 1958; Andrew and Robins, 1969; Demetrio *et al.*, 1972). Nodules often contain 2-3 times more phosphorus per unit dry matter than the roots on which they are formed (Poschenrieder, *et al.*, 1940; Mosse, *et al.*, 1976); the rate of fixation per g of nodule can be greatly increased by phosphorus (Lüdecke, 1941). Various minor elements such as molybdenum, boron, zinc, iron, copper and sulphur are also required for satisfactory growth and/or N_2 fixation.

VA MYCORRHIZA AND PLANT P UPTAKE

Vesicular-arbuscular (VA) mycorrhiza can greatly improve phosphate supply of the host plant by making use of the absorbing capacity of the extensive network of external hyphae associated with the infected root (Mosse, 1973; Gerdemann, 1975; Tinker, 1975). This network extends several cm from the root surface and provides a very effective uptake system drawing nutrients not only from soil beyond the root hair zone but also from outside the depletion zone that develops around roots, particularly for ions that diffuse slowly in the soil. Such ions are notably phosphate but also zinc and molybdate.

The mechanism of nutrient uptake into the soil hyphae is believed to be similar to that operating in the plant root, and soil labelling experiments with ^{32}P (Sanders and Tinker, 1971; Hayman and Mosse, 1972) show that mycorrhizal and non-mycorrhizal plants utilise similarly labelled fractions of the soil phosphate. VA endophytes are in general no more able to mobilise insoluble soil phosphate than plant roots, but Mosse, Hayman and Arnold (1973) reported that mycorrhizal *Centrosema pubescens* took up labelled phosphate from a very P deficient

soil from which the non-mycorrhizal plants took up none. Also VA mycorrhiza can greatly improve growth of plants supplied with relatively insoluble phosphate sources such as bone-meal, tricalcium phosphate and apatite (Daft and Nicolson, 1966; Murdoch, Jakobs and Gerdemann, 1967), rock phosphate (Jackson, Franklin and Miller, 1972; Mosse, Powell and Hayman, 1976) iron and aluminum phosphate (Ross and Gilliam, 1973) and some phytates (Hayman and Mosse, 1972). The extra P was taken up from the soluble P fraction associated with such phosphate sources or released from them in the soil. Mycorrhiza therefore ensure a better utilization of the available phosphate rather than mobilisation of the insoluble fraction. It follows that after a mycorrhizal crop, particularly if it is annual, available P reserves in the soil will be depleted, since insoluble P will only go into solution when the concentration of the soil solution falls below the solubility product of the insoluble P which is likely to be low. Such depletion can be verified by soil analysis at the end of an experiment (Gerdemann, 1964, 1965) or by biological assay with a non-mycorrhizal, P-sensitive plant such as radish (Mosse, 1977b).

Phosphate is stored, and probably transported within the fungal system as polyphosphate granules (Ling-lee, *et al.*, 1975; Cox, *et al.*, 1975). Mycorrhizal roots may thus act as storage organs of applied phosphate absorbed before it becomes immobilised in soil. Transfer mechanisms from fungus to plant are clearly very efficient although not fully understood. Tinker (1975) discusses possible mechanisms of uptake, translocation and transfer in mycorrhizal roots. Because legume nodules are usually not invaded by the fungus, which remains confined to the root cortex, the fungal phosphate has to be released from the fungus into the root and then transferred into the nodule.

EFFECT OF LEGUME SPECIES

In 1944 Asai reported that nodulation in various legume species inoculated with the appropriate rhizobia and grown in soil ("steamed under pressure") depended on the addition of an inoculum of 5-10g of garden soil and the subsequent formation of VA mycorrhiza in the experimental plants. Little was then known about the involvement of mycorrhiza in plant P nutrition and the effects described were attributed to an alleviation of soil toxicity induced by sterilisation. In the light of recent evidence that VA mycorrhiza can affect nodulation (Crush, 1974; Daft and El-Giahmi, 1974, 1975; Mosse, *et al.*, 1976; Mosse, 1977a), Asai's results merit re-consideration. He reported that only four out of 59 legume species examined were not mycorrhizal and Strzemska (1975a) confirms the widespread occurrence of mycorrhiza in twenty species in the Papilionaceae. Some species, e.g. *Trifolium pratense* L., *Vicia sativa* L., and *Trifolium hybridum* L., were consistently strongly mycorrhizal while *Melilotus albus* Desr., *Lotus uliginosus* Schk; *Trifolium repens* L. and *Medicago lupulina* L. were more sparsely infected.

Asai's experiments showed that legume species differed in their

growth responses to mycorrhizal infection. *Mimosa pudica*, and *Cassia tora* grew better when mycorrhizal, but neither mycorrhizal nor non-mycorrhizal plants formed nodules. Growth and nodulation of *Astralagus sinicus*, *Glycine soya*, *Ornithopus sativus* and *Vicia villosa* were much stimulated by mycorrhiza, and without them these plants formed few nodules which had little effect on growth. *Vicia sativa* and particularly *Lupinus luteus* were less dependent on mycorrhiza. Inoculation with rhizobia alone increased their weight by 50-75% and rhizobia + garden soil inoculum increased it by 100-150%. Crush (1974) studied the effects of mycorrhiza on nodulation of four legume species (Table 1). *Lotus pedunculatus*, which had the longest root hairs, grew and nodulated well without mycorrhiza or phosphate, while *Trifolium repens*, *Centrosema pubescens* and *Stylosanthes guyanensis* needed one or other for adequate nodulation. *Stylosanthes* seemed to benefit more from mycorrhiza than from phosphate but the amount of phosphate added led to very high, possibly supraoptimal P concentration (0.58%) in these plants. Generally, effects of mycorrhiza and phosphate supplied in appropriate amounts, are similar. Daft and El-Giahmi (1974, 1975) showed that *Phaseolus vulgaris*, *Medicago sativa* and *Arachis hypogaea* grown in sand with bonemeal as a phosphorus source, grew little and formed few nodules unless plants were also inoculated with a VA endophyte or given soluble phosphate. Bean plants inoculated with *Rhizobium* and a mycorrhizal endophyte, contained 6% more protein in the shoots and more leghaemoglobin per unit nodule fresh weight than those inoculated with *Rhizobium* only.

Lupins are known to be good colonisers of marginal, phosphate-deficient soils, and might therefore be expected to be strongly mycorrhizal. However Asai's (1944) results suggest that their mycorrhiza dependence is relatively small and recent work confirms this. *Lupinus cosentinii* from various sites in W. Australia rarely had more than 10% infected roots, containing virtually no arbuscules (Trinick, 1977). Its seed coat and possibly also its exudates were toxic to two VA fungi (Morley and Mosse, 1976). Nevertheless *L. cosentinii* grew and nodulated well in a very phosphate-deficient soil from which it extracted more than 2000 µgP, exceeding the $NaHCO_3$-soluble P reserves of the soil as determined by the method of Olsen *et al.*,(1954). In the same soil *Stylosanthes guyanensis* grew very badly, took up only 60 µgP and failed to nodulate unless it was mycorrhizal (Table 2). Although many legumes appear to be strongly dependent on mycorrhiza for P uptake, and therefore for nodulation in marginal, P deficient soils, the degree of dependence clearly depends on the species.

In marginal soils interspecies competition for scarce nutrients is likely to occur, and those species best able to extract or supply limiting nutrients will become dominant. Where nitrogen is limiting this is likely to be the nodulated legume fixing nitrogen symbiotically, but if phosphate is limiting those species with the most efficient mycorrhiza or alternative P uptake mechanism, will become dominant. Baylis (1975) considers the development of abundant, long root hairs as an alternative evolutionary trend to mycorrhiza. Temperate grasses are usually better absorbers of phosphate than legumes and therefore

Table 1. Nodulation response to mycorrhiza (M) and monocalcium phosphate (P). (Crush, 1974)

Treatment	Nodulation+ M	Nodulation+ Nil	Nodulation+ P*	Dry wt(g) Nil	Dry wt(g) M	Dry wt(g) P	Root hair length° (μm)
Species							
Stylosanthes guyanensis	5	0	5	0.5	1.6	0.9	108
Centrosema pubescens	5	1	5	1.6	3.9	4.9	106
Trifolium repens	5	1	5	1.6	2.6	4.0	213
Lotus pedunculatus	4	4	5	2.5	2.0	3.9	809

+ Category 0-5, no to abundant nodulation
* 0.1gP/kg soil
° of seedlings grown in a fertile irradiated soil

Table 2. Growth, P uptake and nodulation of two legumes in a Western Australian lateritic soil

	Lupinus cosentinii	*Stylosanthes guyanensis*	
	Mycorrhizal resistant	Non-mycorrhizal	Mycorrhizal
Dry wt (mg)	3442	76	945
P uptake (μg)	2227	65	890
Nodule dry wt(mg)/nodule	83	0	35

tend to outgrow them in P-deficient soils. Temperate grasses do not usually benefit from mycorrhiza until soil P drops below about 4ppm Truog extractable P (Crush, 1973; Powell, 1974). Crush (1974) induced a preferential stimulation of clover grown together with ryegrass in irradiated soil by mycorrhizal inoculation or added phosphate (Fig. 1). A similar, preferential stimulation through improved P uptake occurred when Monterey pine, inoculated with the appropriate mycorrhiza, was grown with Sudan grass (Stone, 1949). The ectotrophic mycorrhizal fungi of pine do not infect the Sudan grass. Some tropical grasses, notably *Paspalum notatum* (Mosse, 1972a, b) but also *Brachearia* and *Digitaria* spp. (Mosse, 1975) are more mycorrhiza-dependent than temperate ones.

EFFECT OF ENDOPHYTE SPECIES

VA endophytes are not host specific although evidence is growing

that certain endophytes may form preferential associations with certain host plants (Mosse, 1975); for instance grasses are often infected with a fine endophyte named *Rhizosphagus tenuis*. Gerdemann and Trappe (1974) proposed five genera in the family *Endogonaceae* of which four contain VA endophytes. They describe 16 species of VA endophytes distinguishable by the structure of resting spores in the soil, but there are also VA endophytes that do not form such spores. Although there is little host specificity the fungi differ in their distribution in different soils (Hayman, 1975), in pH requirements (Mosse, 1972b) in their optimum temperature range (Furlan and Fortin, 1973), and in their symbiotic efficiency as aids in plants P uptake (Mosse, 1975). The efficiency of the mycorrhizal system may be partly dependent on physiological characteristics of the fungi such as translocation rates within and transfer out of the hyphae, but is also likely to depend to a considerable extent on amounts and distribution of the soil mycelium and on interactions between the fungal species and the environment. In this respect the system differs from the rhizobium symbiosis in which effectivity depends more on interactions between rhizobia and the host. VA endophytes have been introduced successfully into unsterilised soils by transplantation of previously infected seedlings (Mosse and Hayman, 1971; Khan, 1972, 1975;) by placing spores, soil sievings or infected roots near seedlings (Redhead, 1975; Janos, 1975) and by pelleting seeds with fresh (Hattingh and Gerdemann, 1975) or lyophilised (Jackson, *et al.*, 1972) sievings. All increased growth of the host plant.

INTERACTIONS BETWEEN MYCORRHIZA, NODULATION AND APPLIED FERTILIZER IN STERILISED AND NATURAL SOILS

Effects of VA inoculation on growth, nodulation and utilisation of added phosphate have been studied in fumigated and natural soils. Mycorrhizal soybeans in fumigated soil yielded 122, 67 and 12% more than non-mycorrhizal in soils given 0, 44 and 176 kgP/ha. They also had larger seeds containing more protein (Ross, 1971). Inoculation also increased soybean yield in one out of two field soils tested but only after fumigation (Ross and Harper, 1970), and it increased yield as well as oil and protein content of the seed in a nodulating but not a non-nodulating isoline of "Hardee" soybeans in a fumigated field soil (Schenck and Hinson, 1973). There was no effect of inoculation on soybean yield in an unsterilised field soil where inoculation improved the growth of corn (Jackson, *et al.*, 1972) and inoculation with either *Rhizobium* and/or *Endogone* did not affect susceptibility of soybeans to *Phytopthora megasperma* or *Phythium ultimum* in laboratory experiments (Chou and Schmitthenner, 1974).

Interactions between VA inoculation, nodulation and rock phosphate have been studied systematically in pot experiments in a series of irradiated and unsterile soils, mostly from Africa, South and Central America (Mosse, Powell and Hayman, 1976; Mosse, 1977a). Table 3 summarises the growth increases obtained in *Stylosanthes guyanensis* by inoculation with a VA endophyte in eleven unsterile soils, with and without added phosphate (Mosse, 1977a). In these experiments the size of the increase was inversely correlated with the extent of mycorrhizal infection by indigenous fungi present in the unsterile soil. Nevertheless VA inoculation also led to some useful growth increases in soils already quite well supplied with indigenous endophytes, possibly because the introduced endophytes were more beneficial or because they were less affected by added phosphate. Added phosphate and nitrogen fertilizers can reduce mycorrhizal infection (Hayman, 1975) but in very poor soils they may increase it by increasing plant growth. Inoculation responses were usually less affected by availability of soil phosphate than by levels of indigenous endophytes but at least one soil contained so little available P that even mycorrhizal plants were unable to extract much extra phosphate from this soil unless rock phosphate was also given. In longer term experiments with larger test plants levels of available soil phosphate may be more important.

In general better growth was associated with better nodulation in both irradiated and unsterile soils (Mosse, Powell and Hayman, 1976; Mosse, 1977a). The best growth and nodulation occurred in plants inoculated with VA endophytes and given rock phosphate, in those soils where non-inoculated plants also showed some response to rock phosphate. Such soils were mainly acidic. In neutral and alkaline soil rock phosphate remained essentially unavailable to both mycorrhizal and non-mycorrhizal plants. The marked growth stimulation of VA inoculated *Stylosanthes* also given rock phosphate persisted into the following

Table 3. Growth increases in *Stylosanthes guyanensis* in eleven unsterile soils inoculated with VA endophytes with and without rock phosphate (after Mosse, 1977a).

No.	Soil from	% infection*	P soluble in: 0.05 NaNCO mg/kg soil	P soluble in: 0.01 M CaCl moles/l.	Growth increase** without rock phosphate M/Nil	Growth increase** with rock phosphate RP+M/RP
1	Isoya, Nigeria	0	4	0.4	x9	x13
2	Isoya, Nigeria	0	3	0.4	x9	x10
3	Ikenne, Nigeria	0	2	1.4	x4.5	x6
4	Ikenne, Nigeria	10	6	0.7	x2	x1.7
5	Ambohinam, Madagascar	10	4	0.4	x1.5	x3.7
6	Netim, Nigeria	15	14	2.1	x1.9	x2.2
7	Egbeda, Nigeria	20	5	0.3	x2	x2.1
8	Bambey, Senegal	50	6	0.8	x1	x3.1
9	Netim, Nigeria	70	5	0.3	x1.3*	x1*
10	Brazil	70	5	0.4	x1	x1.3
11	W. Australia	90	3	0.2	x1.2	x1.4

* indigenous fungi in unamended soil
** introduced endophyte died out

RP=Rock phosphate; M=inoculated with VA endophyte

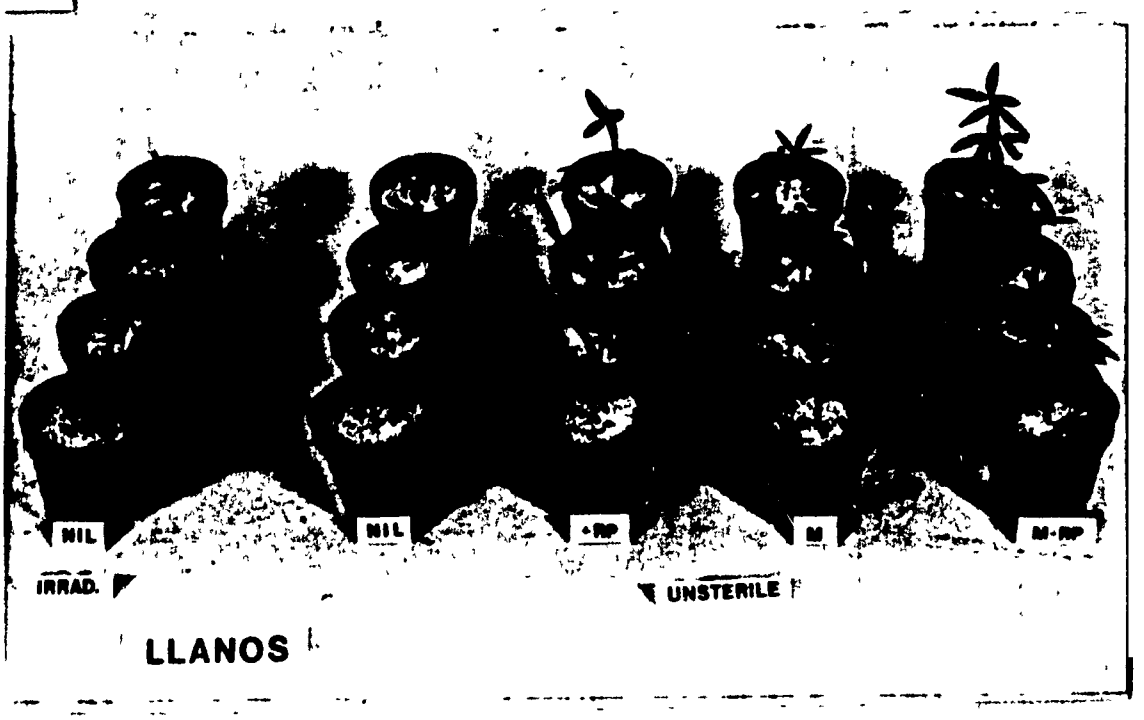

Table 4. Nodule weight and number of mycorrhizal and non-mycorrhizal plants with and without rock phosphate (after Mosse, *et al.* 1976)

Soil	Plant	Unit*	Treatment**			
			Nil	RP	M	RP+M
Cerrado A	*Stylosanthes*	D.W. (mg)	0	0.3	0.7	4.5
" B	"	no.	0	8	6	79
" A	*Centrosema*	F.W. (mg)	0	2	4	208
" A	clover	no.	0	1	3	55
Clay loam, med. forest	*Centrosema*	D.W. (mg)	0	2	79	103
Loamy sand, pasture	"	no.	0	7	-	37
Clay loam, low forest	*Stylosanthes*	D.W. (mg)	7	11	19	15
Sandy loam, agricultural	clover	D.W. (mg)	-	70	-	1050

* Dry weight (D.W.), fresh weight (F.W.), or number (no.) of nodules
** RP=rock phosphate, 100 mg/250 g soil; M=mycorrhizal.

crop, *Centrosema pubescens* and *Trifolium pratense* in an irradiated soil. Without added phosphate however effects of soil depletion by the mycorrhizal *Stylosanthes* were evident in the second crop, particularly the *Centrosema*. Early stages of a good response to inoculation and rock phosphate in an unsterile soil from the Llanos region of Colombia are shown in Fig. 2. This soil contained many spores of indigenous endophytes.

In six out of seven irradiated soils from Brazil, Beliza and England *Stylosanthes, Centrosema and Trifolium* inoculated with the appropriate rhizobia failed to nodulate unless plants were either inoculated with a VA endophyte or given rock phosphate (Table 4). A certain minimum growth and, for *Stylosanthes*, a dry matter P content above 0.1% were needed for nodulation in the irradiated soils. In the unsterile soils this relationship between nodulation and P content was less consistent and some plants containing about 0.1%P nodulated quite well, as shown in Table 5 for two soils from Ikenne in Nigeria. The Nil and RP plants illustrated in Fig. 3 (Soil A) remained small but nevertheless formed some nodules which, however, fixed little nitrogen. VA inoculation greatly improved P uptake, growth and N_2 fixation in the soil (Table 5) without greatly increasing plant P concentration. In soil B (Fig. 4) which contained indigenous rhizobia and some endophytes Nil and RP plants grew better, nodulated well and had good fixation rates. If the indigenous rhizobia were better adapted to low phosphorus conditions than the strain RCR 3831, used as inoculant in all the experiments, this might explain why Nil plants sometimes nodulated well in unsterile soils at low plant P concentrations. Irradiation kills the indigenous rhizobia and nodulation would then depend on the more P-demanding introduced rhizobia (RCR 3831). Selecting *Rhizobium* strains for ability to nodulate at low plant P concentrations may be worth considering.

In one irradiated soil containing at least four times more $CaCl_2$-soluble P than the others (13 μmoles P/L) the Nil plants nodulated, but even in this soil VA inoculation improved P uptake and nodulation. In some soils nodulation was improved by extra P uptake that was no longer reflected in additional growth, i.e. when the plant P content rose above the "critical concentration" for growth (Andrew and Robins, 1969). Inoculation with VA endophytes may lead to such extra P uptake resulting in better N_2 fixation which could in turn improve growth. Nitrogen fixation probably contributed to the growth of plants illustrated in Fig. 4 as the soil contained only 0.06%N.

A similar growth response probably also involving an interaction between the introduced endophyte and nodulation occurred in another soil where all plants at harvest had very similar % P (Soil C Table 5, and Fig. 5). Nil and RP plants became infected by indigenous endophytes in this soil and formed many small nodules, which however fixed very little nitrogen. Inoculation with VA endophytes resulted in better growth, better nodulation and good fixation rates. Responses to rock phosphate were not significant, and appearance of the M and RP+M plants (Fig. 5) suggests that extra nitrogen may have contributed to their good

Table 5. Effects of VA inoculation and rock phosphate on growth and nodulation of *Stylosanthes guyanensis* in three unsterile soils (Mosse, 1977).

Soil A (Ikenne, Nigeria)	Treatment*				L.S.D.**
	Nil	RP	M	RP+M	P = 0.05
Dry weight (g)	0.14	0.20	0.65	1.12	0.40
Total P	0.11	0.22	0.74	1.12	0.27
%P	0.08	0.11	0.12	0.10	-
Nodule dry weight (mg)	0.3	0.2	2	8	-
N_2 fixation C_2H_4/pot/h	negligible		0.37	0.87	0.61
% mycorrhizal infection+	0	0	90	55	
			Introduced		
Soil B (Ikenne, Nigeria)					
Dry weight (g)	0.56	0.91	1.10	1.54	0.39
Total P (mg)	0.54	0.82	1.82	1.32	0.25
%P	0.1	0.09	0.07	0.09	-
Nodule dry weight (mg)	4	5	5	9	2.4
N_2 fixation C_2H_4/pot/h	0.76	0.49	0.21	0.50	0.22
% mycorrhizal infection+	10	5	60	60	
			Indigenous		
Soil C (Netim, Nigeria)					
Dry weight (g)	0.75	0.79	1.39	1.71	0.58
Total P (mg)	0.76	0.90	1.40	1.55	0.50
%P	0.1	0.09	0.1	0.09	-
Nodule dry weight (mg)	approx 40 small nodules		7	12	5.9
N_2 fixation C_2H_4/pot/h	-	-	0.44	0.72	n.s.
% mycorrhizal infection+	15	25	80	80	
	Indigenous		Introduced		

* RP=rock phosphate, 6.6 mg/200g soil; M=with mycorrhizal inoculation;
** n.s.=no significant difference
\+ Indig: indigenous; Introd: introduced endophytes.

growth. In some other unsterilised soils the introduced endophytes also seemed to stimulate nodulation more than indigenous endophytes equally effective at mediating P uptake. In the experiments of Daft and El-Giahmi (1974) beans *(Phaseolus vulgaris)* inoculated with mycorrhiza had nodules 10% heavier than those given phosphate. While the principal effect of mycorrhiza on nodulation is undoubtedly phosphate mediated, mycorrhiza may have other, secondary effects, possibly of a hormonal nature. Asai (1944) attributed such effects to a crystalline substance extracted from cultures of a *Fusarium sp.* isolated from mycorrhizal roots. *Fusarium* is not now considered a mycorrhizal endophyte but the effect of this extract, particularly on legumes, is interesting.

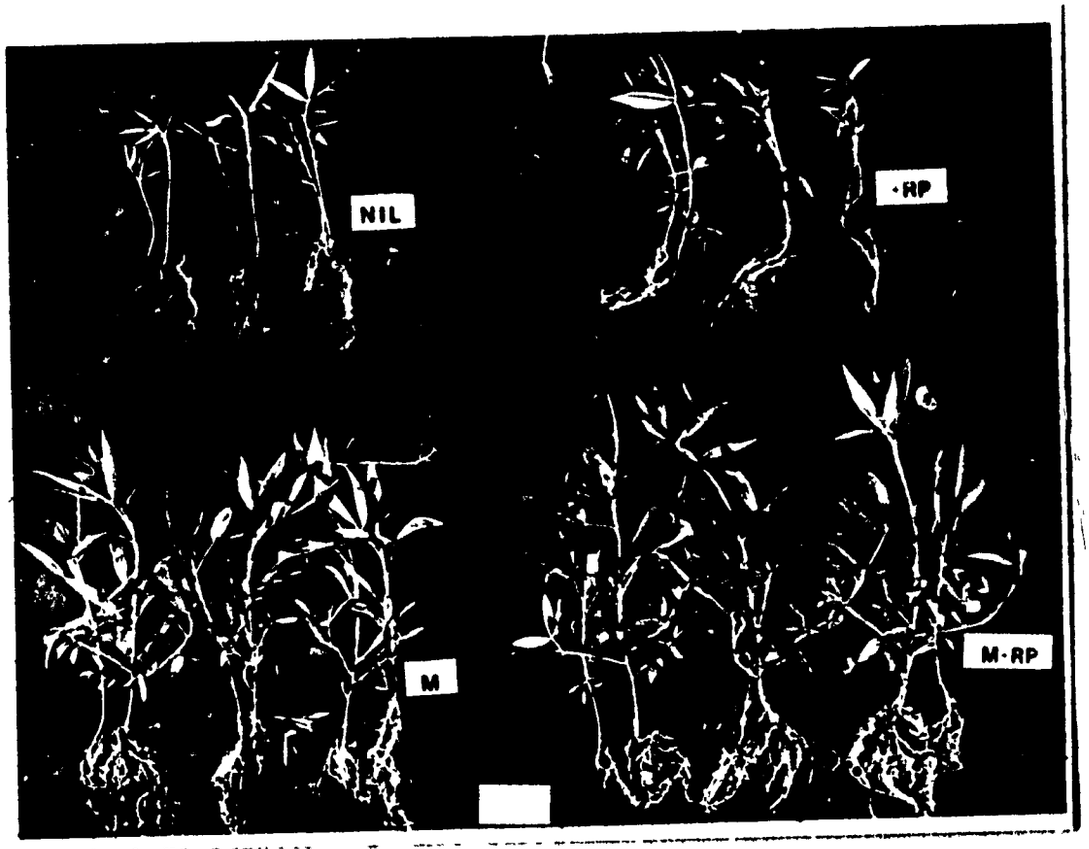

Figure 3. Unsterile soil "A" (Ikenne, Nigeria).

Figure 4. Unsterile soil "B" (Ikenne, Nigeria).

Figure 5. Unsterilized soil "C", table 5 (Netim, Nigeria).

Table 6. Responses to VA inoculation, rock phosphate and monocalcium phosphate in *Stylosanthes guyanensis* grown in two irradiated cerrado soils

Soil 1				
Treatments*	Nil	RP	M	RP+M
Dry wt (mg)	21	99	172	464
Total P (g)	12	108	284	1753
Nodule Dry wt (mg)	0	0.3	0.7	4.5

Soil 2				
Treatments+	Nil	P_1	P_4	M
Dry wt (mg)	36	59	467	217
Total P (g)	38	58	865	443
Nodule Dry wt (mg)	0	0	4.1	0.9

* RP=Rock phosphate at 0.96 mg P/200g soil.
\+ P_1 and P_4=monocalcium phosphate at 5mg P and 20mg P/200g soil.
M inoculated with mycorrhiza.

In some cerrado soils rock phosphate, particularly when coupled with mycorrhizal inoculation, may be a better phosphorus source than more

soluble forms of phosphate (Table 6). Soil 2 contained slightly more available P than soil 1 as shown by the better growth of Nil and M plants in soil 2. Although amounts of monocalcium phosphate added to soil 2 (P_1 and P_4) contained 5 and 20 time as much P respectively as the rock phosphate added to soil 1, RP plants grew better than P_1 plants, and inoculated plants with rock phosphate (RP + M) grew and nodulated as well and took up twice as much P as plants given 20 times as much monocalcium phosphate P (P_4). Some caution is needed in evaluating availability of rock phosphate from experiments in small pots where root growth is restricted within the fertilised zone. However, some very recent results of field inoculations in Nigerian soils (Islam, priv.comm.) show that some of the principles deduced from results of pot experiments also applied in the field. Islam found that : 1) Responses depended on legume species. In the same soil where inoculation increased dry weight of cowpeas by 100% soybeans showed no inoculation response. 2) In appropriate soils inoculation with a selected endophyte increased dry weight of maize and cowpea by 25% and 100% respectively although levels of mycorrhizal infection were similar in inoculated and non-inoculated plots, i.e. the introduced endophyte was more beneficial. 3) Nodule efficiency (nitrogenase activity/unit weight of nodule) was doubled by inoculation of two varieties of soybean while growth was only marginally increased. 4) Similar responses to VA inoculation occurred in pot and field experiments but those in pots tended to be greater. Responses to added rock phosphate in pots were not necessarily repeated in the field.

EFFECT OF MYCORRHIZA ON THE UPTAKE OF OTHER ELEMENTS

The published reports concerning mycorrhizal uptake of elements other than phosphorus are conflicting (Mosse, 1973), but copper which is necessary for nodulation (Hallsworth, 1958) is consistently present at greater concentrations in mycorrhizal plants. Mycorrhiza also assist zinc uptake (Gilmore, 1971) and zinc deficiency following field fumigation has been cured in peaches (LaRue, *et al.*, 1975) and in cotton (Wilhelm, *et al.*, 1967) by mycorrhizal inoculation. In mycorrhizal Pinto bean 18ppm added zinc increased nodulation but further additions depressed both mycorrhizal infection and growth and made the plants more susceptible to ozone damage (McIlveen, *et al.*, 1975). Detached mycorrhizal root segments took up more sulphur-35 from solution than non-mycorrhizal (Gray and Gerdemann, 1973) but Morrison (1962) found no increase in sulphur uptake of ectotrophic mycorrhizal roots of *Pinus*.

It is likely that exudates of mycorrhizal roots differ both quantitatively and qualitatively from non-mycorrhizal and this could affect rhizobia in the soil. More important could be the effect of exudates on free living-nitrogen fixing organisms in the rhizosphere but there is so far no information on exudates of roots with and without mycorrhiza.

AGRICULTURAL APPLICATION

It can not yet be predicted whether, and in what form field inoculation with VA endophytes may become economically practicable. Because the fungi are root - as well as soil inhabiting the difficulties usually encountered in establishing free living organisms in unsterile soils (Brown, 1974) may be less acute. Nevertheless certain soil limitations, such as pH, appear to operate and there are presumably underlying causes for the quantitative and qualitative differences in endophyte populations of different soils. Both fertility levels and crops can affect spore numbers (Hayman, 1975; Strzemska, 1975b; Krucklemann, 1975). Clearly conditions determining indigenous endophyte populations may also affect the establishment of introduced ones, unless existing spore distribution in different soils is entirely fortuitous. Information on rates of spread of introduced endophytes in unsterile soils would be important in determining inoculation patterns. Since numbers of endophyte propagules and infection levels seem to be important determinants of inoculation responses a rapid method of assessing effectivity of indigenous endophytes in their natural surroundings would help to pinpoint sites where field inoculation might be rewarding (Boatman and Paget, in preparation). It is also necessary to confirm that responses obtained in pot experiments are reproducible, even if smaller, in the field where roots may more easily outgrow the introduced endophyte, and added fertilizer will be less well distributed. In two recent reviews (Gerdemann, 1975; Tinker, 1975) and some recent papers (Mosse, 1977a; Daft, *et al.*, 1975) the practical possibilities of field inoculation and their application to special situations have byen considered. Among these are fumigated nursery soils, hill pastures where clover establishment is desired, reclamation sites such as coal tips and sand dunes where legumes both annual and perennial are often important pioneer plants. In considering the potential value of field inoculation with VA endophytes an important distinction should probably be made between annual and perennial crops, because the latter may obtain only a small proportion of their annual phosphate requirement from the soil (Switzer and Nelson, 1972), whereas annuals often have a high P requirement during early growth. Many marginal soils are more suitable for forest than for agriculture. The most important temperate forest species, notably in the *Pinaceae* and *Fagaceae* have ectotrophic mycorrhiza but some leguminous tree species like *Acacia* (Johnson and Michelini, 1974) and *Robinia* (Daft, *et al.*, 1975) also *Alnus*, *Araucaria* (Bevege, 1971) and many tropical tree species (Redhead, 1974) have VA mycorrhiza.

Production of sufficient inoculum is likely to be difficult until ways can be found of culturing VA endophytes. At present they remain obligate symbionts dependent for growth on association with living plant roots. Identification, maintenance and testing of the increasing number of known endophytes require much time and space for which there is at present no central provision. A world bank providing information and starter inoculum for testing the efficacy of field inoculation under

different conditions would greatly assist the conduct of field experiments which are needed to obtain data on which the potential of endophyte inoculation for increased crop production and better fertilizer exploitation can be assessed.

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INOCULATION OF TROPICAL PASTURE LEGUMES

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ABSTRACT

Tropical pasture legumes are grouped into three categories according to their effectiveness response patterns with strains of *Rhizobium*. Three groups are recognized: Group PE which are effectively nodulated by a wide range of strains from many genera; Group PI, nodulated by a range of strains but frequently ineffectively; and Group S, legumes which nodulate effectively only with their own rhizobia.

The likely results from a three-treatment (uninoculated, inoculated and inoculated plus nitrogen) need-for-inoculation trial are tabulated and discussed, and their interpretation related to selection of strains of *Rhizobium* for legume inoculant preparation.

Inoculation of seed is described and the value of using the peat form of the inoculum and pelleting with either lime or rock phosphate is discussed in relation to survival of the inoculum on the seed, multiplication in the rhizosphere, and adverse (acid) soil conditions.

Root temperatures of approximately 30°C are indicated as optimal for both nodulation and nitrogen fixation in tropical pasture species.

INTRODUCTION

The search in recent years for supplies of nitrogen, other than chemically-produced fertilizer nitrogen, for protein synthesis has increased research into biologically fixed nitrogen and in particular that of the legume *Rhizobium* symbiosis. In this role the tropical pasture legume can play an important and increasing part in augmenting protein supplies in tropical areas (Dawson, 1970). Nitrogen supply by the legume is dependent on formation of nodules by strains of *Rhizobium* effective in nitrogen fixation. Where such strains do not occur naturally in the soil they can be provided by seed inoculation and where strains occur naturally these must be evaluated for ability to fix nitrogen. Thus, if a legume is introduced into a pasture system for the first time or reintroduced as part of a ley improvement program it is necessary to have some knowledge of its requirements for formation of effective nitrogen fixing nodules; that is, does the legume

(a) have a special strain requirement or will it nodulate effectively with a range of strains of *Rhizobium* and (b) do such strains exist in the location where the legume is to be grown or must they be supplied by inoculation of seed with a selected and proven strain.

The increasing use of pasture legumes and the wide range of species and cultivars available for use in different agronomic situations has highlighted many gaps in our understanding of *Rhizobium* strain requirements for effective nitrogen fixation response. These responses range from species which nodulate effectively with a range of rhizobia from many different legumes, e.g. *Macroptilium atropurpureum* (nodulation group PE), to the very strain specific *Lotononis bainesii*, which nodulates effectively only with its rhizobia (nodulation group S). Between these extremes there is a whole suite of legumes whose responses to rhizobia are intermediate in that they can nodulate with a large number of strains of rhizobia but are frequently only partially effective or wholly ineffective in nitrogen fixation (nodulation group PI). Furthermore there are many gaps in our knowledge of the effects of environmental factors such as soil temperature, soil pH, soil chemical status and plant nutritional requirements on nodulation and nitrogen fixation.

This presentation will concentrate on the applied aspects of obtaining effectively nodulated pasture legumes and will consider host groupings based on effectiveness of *Rhizobium*, the need for inoculation, criteria for selection of strains, and some of the important factors affecting survival of rhizobia on the inoculated seed and nodulation of the seedling host.

INOCULATION GROUPS

The three major groups (PE, PI and S) of tropical pasture legumes recognized above can be divided conveniently into sub-groups, also, on the basis of their nitrogen-fixing effectiveness response to a range of strains of *Rhizobium*. The degree of specificity increases from group PE to PI and group PI to S (Table 1). These groupings are not necessarily correlated with taxonomic groupings and may have little relation to the ability of strains of *Rhizobium* to form nodules. It is intended as a working guide for inoculum strain recommendations and legume inoculant preparation.

The unspecialized cowpea-type *Rhizobium* (Norris, 1956) is so widespread in tropical soils (Norris 1969, 1972a) that legumes of group PE rarely respond to inoculation (e.g. Bowen, 1956b; Lopez, *et al.*, 1972; Date, 1973 and unpublished) but are nodulated effectively by naturally occurring soil rhizobia from a range of leguminous genera (Allen and Allen, 1939; McKnight, 1949; Bowen, 1956a,b; Norris, 1972a). The effective response patterns in this group, although very broad, may differ from species to species (Date, unpublished).

Despite their broad response patterns nodulation failures may occur because of low numbers of rhizobia in the soil (Graham, unpublished; Date, unpublished) or because low soil pH limits multiplication of the few rhizobia present in the soil (Munns, 1965; Morales, *et al.*, 1973).

Table 1. Inoculation groups for tropical pasture legumes based on effectiveness of nitrogen fixation with a range of strains of *Rhizobium*

GROUP PE Nodulate effectively with a wide range of strains (=Old Cowpea-type group). Genera listed forming one loose group.

Arachis	*Lablab*	*Gliricidia*
Calopogonium	*Macroptilium*	*Psophocarpus*
Cajanus	*Macrotyloma*	
Canavalia	*Pueraria*	
Clitoria	*Rhynchosia*	
Crotalaria	*Stizolobium*	
Cyamopsis	*Stylosanthes* (several sub-groups)	
Desmanthus	*Teramnus*	
Dolichos	*Tephrosia*	
Galactia	*Vigna*	
Glycine	*Zornia*	
Indigofera		

GROUP PI Nodulate with a range of strains but often ineffectively. Genera listed forming individual groups with some crossing between groups. Sub-groups distinguishable.

Centrosema (2 sub-groups)	*Adesmia*
Desmodium (2 sub-groups)	*Aeschynomene*
Stylosanthes	*Psoralea*
	Sesbania (2 sub-groups)

GROUP S Nodulate effectively with specific strains only. Genera listed forming specific groups.

Cicer	*Coronilla*
Leucaena	*Lotus*) 2 sub-groups
Lotononis - *Listia* (minimum 3 sub-groups)	*Lupin*)
Medicago - *Trigonella*	
Mimosa	
Stylosanthes (2 sub-groups)	
Trifolium (African) (4 sub-groups)	

High soil temperatures, low soil moisture content and nutritionally unfavourable conditions restrict multiplication of existing soil rhizobia but their effects are not well defined. The absence of a suitable host plant (rhizosphere) also limits multiplication.

Legumes of group PI are the most troublesome since they nodulate with many strains of *Rhizobium*, but are effective in nitrogen fixation with only a limited number of them. Thus inoculation and nodulation failures are more frequent because the inoculum strain is unable to compete with the ineffective but established soil populations of rhizobia e.g. *S. guianensis* cv Oxley (Date, unpublished). This cultivar

has an apparent specific requirement since it nodulates ineffectively with many existing soil rhizobia but is grouped with other promiscuously nodulating *S. guianensis* lines when screened against a range of rhizobia of different species and country origins (Date and Norris, unpublished). *Desmodium* species demonstrate varying specificities and commonly require inoculation (Diatloff, 1968; Diatloff and Luck, 1972). *Desmodium intortum, D. barbatum,* and *D. tortuosum* are more freely nodulating than *D. uncinatum, D. distortum, D. heterophyllum* and *D. cuneatum* (Diatloff, 1968). *Desmodium heterophyllum* is not effectively nodulated by the strain CB627 which is commonly used as an inoculum strain on other *Desmodium* species, but neither is it as specific in its requirement as suggested by Graham and Hubbell (1975). In effectiveness screening trials (Date and Norris, unpublished) this host nodulated effectively with 14 of 40 isolates from 19 species of *Desmodium* and was effective with 5 of 12 from *D. intortum* and *D. uncinatum*. Isolates from 17 nodules formed on *D. heterophyllum* plants grown from uninoculated seed in North Queensland and 2 from Fiji were all effective in glasshouse screening. Other examples include *Centrosema* species (Bowen, 1956a,b; Bowen and Kennedy, 1961), some *Stylosanthes* species (Date and Norris, unpublished; 't Mannetje, 1969; Edye *et al.*, 1974), and *Sesbania* (Johnson and Allen, 1952).

The very specific legumes (group C) generally respond to inoculation and present no inoculation problems provided adequate numbers of rhizobia are applied at sowing. These specificities apply especially when the legume is being introduced into new areas. Species such as *Leucaena leucocephala,* which are usually grouped as specific (Trinick, 1968) might well belong to group PI since in areas such as New Guinea, Fiji Islands, Solomon Is., Hawaii and many other areas where it has spread naturally it nodulates effectively without addition of selected strains. Similarly *Glycine max* needs inoculation only when sown into new areas not previously sown to that legume. Absolute specificities are demonstrated by *Lotononis bainesii* and *Trifolium semipilosum* in the Australian environment. No doubt in East and Southern Africa these species would not respond so readily to inoculation. They are, however, nodulated only by specific rhizobia.

THE NEED FOR INOCULATION

An understanding of the likely nodulation response of a new legume is essential for its successful introduction. It is recommended, therefore, that simple need-for-inoculation trials be carried out in any new situation. Such trials also provide an indication of the criteria to be used for *Rhizobium* strain selection trials. A simple three treatment experiment, similar to, but simpler than that described by Vincent and Nutman for the IBP feasibility trials (Nutman 1976), can provide the necessary information. The three treatments are (a) an uninoculated control, to check for the presence or absence of native rhizobia and their effectiveness; (b) an inoculated treatment using a strain of

Table 2. Possible results from a 3 treatment inoculation trial to determine need to inoculate legume seed

Situation	Uninoculated		Inoculated		Inoculated + Nitrogen	
	Nodulation	Plant Growth	Nodulation	Plant Growth	Nodulation*	Plant Growth
1	-	Poor	-	Poor	-	Poor
2	-	Poor	-	Poor	-	Good
3	-	Poor	+ E	Good	+ or -	Good
4	-	Poor	+ I	Poor	+ or -	Good
5	+ I	Poor	+ E	Good	+ or -	Good
6	+ I	Poor	+ I	Poor	+ or -	Good
7	+ E	Good	+ E	Good	+ or -	Good

- = plants not nodulated E = effective nitrogen fixation
+ = plants nodulated I = ineffective nitrogen fixation
* Plants may or may not be nodulated depending on their sensitivity to nodule formation being inhibited by applied nitrogen.

Rhizobium known to be, or at least reported to be, effective with the host in question, and (c) an inoculated treatment as above plus nitrogen to ensure satisfactory growth of the host.

The combination of results most likely to be observed and their interpretation have been discussed previously (Date, 1976a) but are repeated here and in Table 2.

1. No nodulation and plants poorly grown; small and with severe nitrogen deficiency symptoms in the uninoculated plants, indicating that native rhizobia, suitable for the test legume, are not present in the soil; similarly no nodules and no growth response from either the inoculated or inoculated plus nitrogen treatment indicating that some factor other than nitrogen e.g. phosphorus supply, is limiting plant growth.
2. No nodulation and poor growth of uninoculated and inoculated plants as above but healthy well grown plants with added nitrogen, indicating that either the inoculum strain or the quality of the inoculant supplied was unsatisfactory, or that soil conditions were adverse for growth of the rhizobia and infection of the root.
3. No nodulation and poor growth of the uninoculated plants as before, but effective nodulation of inoculated plants with growth similar to that in the plus nitrogen treatment, indicating success of the inoculum strain in forming nodules and fixing nitrogen.
4. No nodulation and poor growth of uninoculated plants but inoculated treatment plants nodulated ineffectively and growth poor compared to the plus nitrogen treatment, indicating that the inoculum strain was unsuited to the test legume.

5. Uninoculated plants poorly grown and with ineffective nodules indicating nodulation by an unsuitable native strain; inoculated plants effectively nodulated and well grown demonstrating that the inoculum strain was both competitive with the native strains for nodule sites and effective in nitrogen fixation with the test host.
6. Uninoculated and inoculated treatment plants nodulated ineffectively and poorly grown in comparison with the plus nitrogen treatment, suggesting either that native rhizobia were ineffective and competitive for nodule sites, thus keeping out the inoculum strain, or that the inoculum strain was ineffective in nitrogen fixation. It would be necessary in this case to use some strain identifying technique such as serology (see Vincent, 1970) or antibiotic resistance marker (Obaton, 1971) to determine the proportion of nodules in the inoculated treatment due to the applied inoculum strain.
7. Uninoculated plants with effective nodules and well grown compared with the other two treatments, indicating that native rhizobia suitable for the host legume are present in the soil. Obviously there has been no response to inoculation in this situation and nodules on the plants in the inoculated treatment would most likely be due to native strains. Again this could be checked by serological or antibiotic marker identifying techniques. Under rare circumstances it is possible that the inoculated treatment in this situation could be nodulated and poorly grown, indicating a highly competitive but ineffective strain in the inoculated treatment.

Such trials as these can be replicated and additional treatments super-imposed. For example an experiment could consist of 3 replications of each of the treatments described above, with main plots split for phosphorus application. It must be remembered that these experiments presume a basal application of fertilizer and general husbandry of the plants to levels consistent with good plant growth in so far as they are known.

Based on the information obtained from such an exploratory trial the researcher will be able to determine the criteria needed to select suitable strains of *Rhizobium* for each particular host legume and area in which it is grown. For example if results such as in 3 and 5 are obtained then the only criterion needed for strain selection will be effectiveness in nitrogen fixation with the host in question. Or, if the situation is like 6 then obviously competitive ability for nodule formation will have to be added as a criterion in selection even at the expense of having to accept a strain of lower nitrogen fixing ability. Alternatively if the situation is as in 7 then clearly inoculation is not essential and the selection of efficient strains of *Rhizobium* for that situation would be wasted effort.

So far the discussion has dealt only with the need for inoculation in the year of establishment. This is satisfactory for annual legume

crops, but for perennial species persistence of the inoculum strain will also be a characteristic required in suitable strains of *Rhizobium*. The same experiment as outlined above can be maintained and sampled in subsequent years for yield and nodule composition with respect to *Rhizobium* strain. For experiments with annual legumes plots can be resown each year with uninoculated seed. Results for subsequent years similar to situations 2, 3 and 4) would be readily interpreted as failure or success of the inoculum strain to persist. Situations 5, 6 and 7 would be more difficult and may require some interpretation from plant growth (dry matter or N fixed) data and from the proportion of nodules formed by the inoculum strain to ascertain persistence and competitive ability of the inoculum strain. If growth in uninoculated controls in situations 5 and 6 remains poor and plants are ineffectively nodulated, interpretation is the same as for the establishment year. However, experience has shown that growth and nodulation effectiveness in uninoculated controls can improve with year until plants are as well grown as in the inoculated treatment. It seems that in these situations there has been an increase in the proportion of native strains effective in nitrogen fixation, which, in the presence of the host, have developed to a high level in the rhizobial population and are able to compete strongly with the inoculum strain for nodule sites. The inoculum strain correspondingly accounts for fewer and fewer nodules each year. A good example of this situation was obtained at one of our experimental sites in south-east Queensland with *Desmodium intortum* cv. Greenleaf (Table 3). Here inoculation was essential in the establishment year for good plant growth (compare relative growth response between uninoculated and inoculated in year 1). In subsequent years relative growth in the uninoculated treatments continued to increase until it equalled the inoculated. At the same time there was a corresponding decrease in the proportion of nodules due to the inoculum strain in the inoculated treatment. Nitrogen fixing effectiveness tests of strains of *Rhizobium* isolated in the 4th year from plants in the uninoculated treatment indicated that they were as effective as the original inoculum strain.

Table 3. Success and persistence of inoculum strains for *Macroptilium atropurpureum* and *Desmodium intortum* (Date, unpublished.)

	% nodules due to inoculum strain (relative yield*)			
	M. atropurpureum cv. Siratro		*D. intortum* cv. Greenleaf	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Year 1	0 (8)	1 (10)	0 (1)	85 (10)
2	0 (10)	2 (10)	8 (7)	67 (10)
3	0 (10)	1 (10)	0 (10)	31 (10)
4	-	-	13 (10)	17 (10)

* 1 (poorest) to 10 (best) ranking

CRITERIA FOR SELECTION OF STRAINS

The next phase of any development program will involve the selection of strains of *Rhizobium*. Details of methods and criteria have been published on several occasions (Brockwell *et al.*, 1968; Roughley, 1970; Date, 1976b; Norris and Date, 1976). For convenience and streamlining of work programs, criteria can be grouped as follows:

1. Effectiveness in nitrogen fixation: Clearly a strain must fix nitrogen with its recommended host; if not then there is little point in considering it further. Large collections of strains can be successfully screened in controlled environment or glasshouse conditions with confirmation of final selections in the field.
2. Competitive ability in the rhizosphere and in nodule formation (Gibson *et al.*, 1976; Franco and Vincent, 1976; Cloonan and Vincent, 1967; Dobereiner, 1971) and survival in the presence or absence of a host plant (Chatel, Greenwood and Parker, 1968).
3. Characteristics for special circumstances. For example, soil pH as in the case of *Leucaena leucocephala* (Norris, 1973a), fungicide tolerance (Kecskes and Vincent, 1973; Afifi *et al.*, 1969; Diatloff, 1970a, b,), promptness to form active nitrogen fixing nodules at high (tropical bare soil) or low (alpine) temperatures (Brockwell, Bryant and Gault, 1972) and ability to form nodules in the presence of high levels of soil nitrogen (Gibson *et al.*, 1971; Herridge and Roughley, 1975).
4. This group includes all those characteristics related to culturing the strain, its growth and survival as peat culture and its ability to survive on inoculated seed.

For the new investigator there are three further aspects of *Rhizobium* strain selection worthy of mention. Firstly, effectiveness specificity is a host genotype interaction with the invading strain of *Rhizobium*. There are, therefore, two sources of variation: the plant and the strain of *Rhizobium*. Regrettably, the legume bacteriologist is frequently confronted with a plant that has already been selected and for which he must find a suitable strain, thus leaving only variability of strains for exploitation. The range of specificity or host x strain interaction is well illustrated in the African clovers (Norris and 't Mannetje 1964) and in *Desmodium* (table 4, Date and Norris unpublished) where three strains (set 1) were effective (E) on *Desmodium intortum* and ineffective (I) on *D. uncinatum*, while a second three strains (set 2) were ineffective on *D. intortum* and effective on *D. uncinatum*. A third set (set 3) was effective on both hosts. There was no obvious relation between host or country of origin of strain and effectiveness. The second aspect is that there are no good guidelines as to sources of suitable strains. The legume bacteriologist needs to collect strains from other laboratories and make isolations from nodules collected and imported with the plants and from nodules formed by native strains when uninoculated seed is sown into new areas. The third aspect is the

Table 4. Host by strain of *Rhizobium* interaction for *Desmodium intortum* and *D. uncinatum* expressed as percentage of dry weight of best strain for each host species

Host Species	Uninoc. Cont.	Strains of *Rhizobium*									Best strain	+N Cont.
		Set 1			Set 2			Set 3				
		CB1518	CB1627	CB1709	CB1564	CB1789	CB1771	CB934	CB792	CB1517		
D. intortum	0.2	35	71	100	7	9	19	39	53	79	100	72
D. uncinatum	1.3	6	5	25	44	39	71	85	60	77	100	56
Country of origin		Brazil	Brazil	W. Indies	Brazil	S. Africa	Brazil	Brazil	Mexico	Australia		

dilemma of (a) numerous inoculants each with a highly effective strain for individual species or (b) wide-spectrum strains that vary from good to excellent in nitrogen fixation on a range of legumes. Multiple strain inoculants must be avoided because of possible antagonistic and inhibitory effects in culture (Marshall, 1956; Schwinghamer, 1971; Schwinghamer and Belkengren, 1968) and the likelihood of competition for nodule formation from less effective strains (Caldwell, 1969).

FACTORS AFFECTING INOCULATION, NODULATION AND N-FIXATION

The inoculum must provide a high number of viable rhizobia to ensure early nodulation of the young seedling. Experience over recent years has shown that the form of the inoculum is the most important factor in achieving this since even under satisfactory conditions there are large differences in the number of rhizobia surviving on the seed. Broth, agar and reconstituted lyophylized cultures do not survive on the seed as well as the peat form of the inoculum (Date, 1970; Vincent, 1971 and 1974). Pelleting (or coating) with either lime, rock phosphate or bauxite (Date, 1968, 1970; Date and Cornish, 1968; Cass-Smith and Goss, 1964; Norris, 1971a,b, 1972b, 1973b) provides added protection if inoculated seed needs to be stored for short periods before sowing or if sown in contact with acid or alkaline fertilizers (Goss and Shipton, 1965; Date, *et al.*, 1965; Norris, *et al.*, 1970; Hastings and Drake, 1962) or into acid soils (Franco, *et al.*, 1970). Lime coating improves survival on the seed for the fast growing strains typical of clovers, medics and *Leucaena* (Norris, 1973a; Norris, *et al.*, 1967; Date 1965, 1968; Radcliffe, *et al.*, 1967) and their multiplication in the rhizosphere (Hely, 1965; Date, 1968; Cloonan and Vincent, 1967), but is without effect or harmful for the slower growing rhizobia typical of *Macroptilium, Desmodium, Centrosema* and *lupin* (Norris, 1971a,b, 1972b, 1973b; Date and Cornish, 1968; Shipton and Parker, 1966; Herridge and Roughley, 1974). However, in an acid soil nodules were formed on *Centrosema pubescens* with a lime pellet but not with rock phosphate (Dobereiner and Arnovich 1965; Franco,*et al.*,1970).

For most of these legumes it is inherently safer to use a relatively inert coating material such as rock phosphate or bauxite when pelleting except in situations of acid soils or fertilizers. For normal sowings, equally good nodulation can be obtained by inoculating with half strength adhesive (Norris, 1971a, b, 1972b, 1973a, b; Herridge and Roughley, 1974) to ensure complete adhesion of the inoculant during the handling and drilling operations.

The rationale behind the use of either lime or rock phosphate is based on Norris' (Norris 1956, 1967) concept that the legume/*Rhizobium* symbiosis originated in the wet tropics under low pH, leached soil conditions where the legumes adapted to soil of low calcium status by developing an ability to extract their Ca requirement, and the associated *Rhizobium* developed the alkali producing characteristic as a survival mechanism. As legumes have moved away from the tropics and

Table 5. Pelleting guide (from Norris and Date, 1976)

Genera and species with acid-producing *Rhizobium*		Genera and species with alkali-producing cowpea-type *Rhizobium*	
Pellet with lime		Pellet with rock phosphate	
Adesmia	*Pisum*	*Acacia*	*Lespedeza*
Astragalus	*Psoralea*	*Alysicarpus*	*Lotononis*
Cicer	*Sesbania*	*Arachis*	*Lotus pedunculatus*
Coronilla	*Trifolium*	*Calopogonium*	*Lupinus*
Hedysarum	*Trigonella*	*Cajanus*	*Macroptilium*
Lathyrus	*Vicia*	*Centrosema*	(= *Phaseolus*)
Lens		*Clitoria*	*Macrotyloma*
Leucaena leucocephala		*Crotalaria*	(= *Dolichos*)
Lotus corniculatus		*Cyamopsis*	*Ornithopus*
Medicago		*Desmodium*	*Phaseolus* (other than
Melilotus		*Dolichos*	*P. vulgaris* and
Neptunia		*Flemingia*	*PP. coccineus*
Onobrychis		*Glycine*	*Pueraria*
Phaseolus coccineus		*Indigofera*	*Stizolobium*
Phaseolus vulgaris		*Lablab* (=*Dolichos*)	*Stylosanthes*
			Vigna
			Zornia

into non-acid soils the selection pressures changed so that plants adapted to alkaline soils now have an associated *Rhizobium* producing acid as a survival mechanism. Thus most tropical legumes of today represent the original legumes and their slow growing alkali-producing rhizobia represent the archetype nodulating organism. There are of course, exceptions to this general rule, where a plant has adapted to a special environmental niche. For example, the tropical legume *Leucaena leucocephala* has a normal habitat in alkaline soils and is nodulated by fast-growing acid-producing strains of *Rhizobium*. Conversely *Lotus pedunculatus* and some members of the genus *Lupinus* are temperate species adapted to acid soils and require slow-growing alkali-producing rhizobia. A rule-of-thumb guide, when pelleting is necessary, is to use lime pelleting on species which have fast-growing acid-producing *Rhizobium* and rock phosphate (or bauxite) on species with slow-growing alkali-producing *Rhizobium* as suggested in Table 5.

Inoculating and pelleting instructions are very similar (Cass-Smith and Goss, 1964; Roughley, *et al.*, 1966; Diatloff, 1971). The simplest form (or single step) pellet is prepared by mixing the peat with the adhesive which is then used to wet seeds by stirring until all seeds are evenly covered. The required amount of coating material is then added to the seed all at once, and mixed with a tumbling action until all seeds are evenly coated and separated. Excessive mixing will damage the pellets. Small lots are best mixed by swirling in a figure-8 motion in a cylindrical beaker or can. Large scale operations

require the use of a mixing device. Different sized seeds require different amounts of sticker and coating material, and the worst mistake that can be made is to add excess adhesive which will result in the seed aggregating into large gluey lumps when the coating material is added. As a general guide the following quantities are recommended:

The use of 200 ml of adhesive (either 40% w/v gum arabic or 4% w/v methyl cellulose) plus 2.5 kg lime or rock phosphate will be sufficient for 5 kg of small seed (white clover, lotononis), 7.5 kg of small-medium seed *(Desmodium, Stylosanthes)*, 10 kg of medium seed (glycine, Siratro, subterranean clover), or 20 kg of large seed (cowpea, *Lablab purpureus*, leucaena).

The coating materials for pelleting must be ground very finely: 100% less than 44µm and preferably 10 to 20 µm. The finer the particle size the firmer the coating. As adhesives, gum arabic or methyl cellulose have proven very satisfactory (Date 1965, 1968, 1970; Hastings and Drake, 1962; Brockwell, 1962) but where these are unavailable other cellulose derivatives and adhesive agents though slightly inferior are satisfactory (Date, 1965; Graham, Morales and Cavallo, 1974).

In addition to the use of peat culture and seed pelleting for successful nodulation it is important to:

1. Ensure that seed has not been treated with chemicals and that containers used for inoculation are free of toxic substances such as oil, petrol, pesticides, etc. Many of the larger seeded legumes are fungicide treated so that it is important to avoid toxic materials based on copper and mercury. Preparations such as thiram and dexon can be safely used (Kecskes and Vincent, 1973; Afifi *et al.*, 1969; Diatloff, 1970a,b).
2. Keep the inoculant in cool storage until used. High temperatures have a significant effect on survival in peat culture. The effect is worse in non-sterile culture than in pure (sterile) culture (Date, 1959). Strains of *Rhizobium* differ in their ability to grow and survive at different temperatures (Roughley and Vincent, 1967).
3. Sow inoculated seed as soon as possible after inoculation. Pelleted seed should not be stored for more than 2 weeks for clovers, medics and *Leucaena* or 4 weeks for *Desmodium, Macroptilium, Glycine*, etc. Survival on the seed is influenced by the form of the inoculum pelleting treatments, temperature of storage and host species as already discussed (Date, 1970; Vincent, 1971, 1974; Herridge and Roughley, 1974).
4. Avoid mixing inoculated seed with fertilizers (see earlier discussion).
5. Sow into moist soil. The earlier seeds germinate and seedlings become established the greater the chance of an early nodulation and final plant establishment.
6. Use only tested and approved cultures. This will minimize problems associated with poor quality inoculants.

In recent times various other forms of inoculant and seed inocula-

tion have been tried (Porter *et al.*, 1960; Fraser, 1975; Herridge, *et al.*, 1974; Mullet *et al.*, 1974; Brockwell and Hely, 1962). In general all preinoculation treatments such as these have been less than satisfactory (Date and Decker, 1962; Brockwell and Roughley, 1967; Brockwell, *et al.*, 1975; Thompson, Brockwell and Roughley, 1975). Solid, pelleted or prilled inoculum as granules (Frazer, 1975; Burton pers com; Brockwell pers com; Roughley pers com) of various types mixed and sown with seed have proven successful, especially where seeds are pesticide treated. These methods, however, have more application to the grain legumes than to pasture legumes. An extruded pellet (Philpotts pers com) containing seed and inoculum has given good results in preliminary evaluation.

The influence of plant nutritional restraints on nodulation and nitrogen fixation were discussed by Dr. C.S. Andrew (Session 3 this workshop). The effects of soil acidity and calcium supply, phosphorus, sulphur, molybdenum, cobalt, soil nitrate and moisture, and manganese and aluminum toxicity on nodulation of tropical legumes were reviewed briefly by Norris and Date (1976). The effect of the mycorrhizal association of *Endogone* and legumes on phosphorus supply and nodulation was discussed by Dr. B. Mosse (session 3, this workshop).

There are few critical studies on the effects of root temperature on nodule formation and nitrogen fixation in tropical pasture legumes; however, it is clear that many of them have temperature optima near 30°C. For example, growth and N-fixation in *Glycine wightii* (Ferrari, *et al.*, 1967) *Stylosanthes guianensis* and *Pueraria javanica* were reduced under daily maximum temperatures of 34-43°C compared with 29-32°C (Souto and Dobereiner, 1968, 1970); reduced nodulation, nitrogen fixation and forage yield in *Centrosema pubescens* at soil temperatures above 30°C (Dobereiner and Aronovich, 1965); and the nodulation of *G. wightii*, *Desmodium uncinatum*, *D. intortum*, *Stylosanthes humilis* and *Macroptilium atropurpureus* in terms of number of nodules formed and number of plants nodulated was less at 24 and 36°C than at 30°C (Gibson, 1971). In controlled environment, at constant shoot temperature, the optimum root temperature for nitrogen fixation for six lines of *Stylosanthes* was near 30°C also (table 6, Date, unpublished).

Although tropical legumes appear to have higher optimum temperatures, than temperate species, for nodulation and nitrogen fixation, their associated rhizobia are not necessarily more temperature tolerant. The upper limit for growth of 68 strains from several cowpea-type species (*Vigna*, *Crotalaria*, *Arachis*, *Glycine*, *Indigofera*), *Centrosema*, *Desmodium*, *Sesbania* and *Pultenaea* ranged from 30-42°C and was little different to that for 8 strains from medics (36.5-42.5°C) and 11 strains from clovers and peas (31-38.4°C) (Bowen and Kennedy, 1959). Ishizawa (1953) also reports that there is little difference in maximum temperature for growth between rhizobia from temperate or tropical legumes. He noted as did Bowen and Kennedy (1959) that the maximum temperature for growth of slow-growing strains was slightly lower than forsome fast-growing strains. Thus in soils where temperatures frequently exceed 45°C and 50°C for several hours daily, rhizobial populations could be severely reduced with consequent depression of nodulation . There are some indications

Table 6. Nitrogen fixation (mg N/plant) by six lines of *Stylosanthes* at six root-temperatures during days 21 to 42 of growth in controlled environment at 30°C shoot temperature (Date, unpublished)

Root Temp. °C	*S. hamata* Verano*	*S. hamata* CP140264A+	*S. guianensis* Endeavour*	*S. humilis* Lawson*	*S. scabra* CP140292+	*S. fruticosa* CP141219A+
15	0.9	1.0	1.4	1.7	0.8	1.3
20	1.7	2.0	3.8	3.9	1.8	2.3
25	3.6	3.4	4.3	4.8	2.9	4.2
30	6.0	4.6	4.8	5.3	3.3	4.5
35	5.9	4.1	4.5	4.8	1.7	3.6
40	1.8	0.2	1.1	1.0	0.3	1.2

* cultivar
\+ Commonwealth Plant Introduction (CPI) number

however that in hot dry soils clover (Essawri and Abdel-Ghaffar, 1967) and cowpea-type (Wilkins, 1967) rhizobia may adapt to elevated temperatures. Similar exposures in moist soil for even short periods killed all strains (Wilkins, 1967).

AMOUNTS OF NITROGEN FIXED

The amount of nitrogen fixed by tropical pasture legumes is variable and estimates range from 20 to 180 kg N $ha^{-1}yr^{-1}$ if allowances are made for losses and transfers and N taken up from the soil, as Henzell (1968) calculated for a range of tropical legumes. Values of 108 kg N $ha^{-1}yr^{-1}$ (Bruce, 1965) to 280 kg N $ha^{-1}yr^{-1}$ (Moore, 1962) have been reported for *Centrosema pubescens* and as high as 580 kg N $ha^{-1}yr^{-1}$ for *Leucaena leucocephala* (Hutton and Bonner, 1960) when based on nitrogen content of dry matter yields of tops. Estimates for other species and situations have been reviewed in several places (e.g. Henzell and Norris, 1962; Date, 1973; Vincent, 1974).

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INOCULATION AND NITROGEN FIXATION IN THE GENUS *PHASEOLUS*

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Though there is no agreement on the number of *Phaseolus* species and the characteristics of each (Kaplan, 1965; Purseglove, 1968), four agriculturally important species are normally recognized (Miranda, 1966; Smartt, 1970). These are *P. vulgaris* L, *P. coccineus* L, *P. lunatus* L, and *P. acutifolius* Gray.

As we have obtained only limited information on the latter three species, and virtually nothing on lesser species such as *P. adenanthus* or *P. ritensis*, this review will emphasise *P. vulgaris*. Despite the limited *Phaseolus* bibliography we have resisted the temptation to draw heavily on more studied genera; nor do we consider Asian species such as *P. mungo* and *P. aureus*, transferred to the genus *Vigna* following detailed studies by Verdcourt (1970).

The agriculturally important *Phaseolus* are predominantly annuals, bush or climbing in growth habit, and with a wide range of seed colours, sizes and shapes (Fig. 1), flowering and maturity times and disease resistance capabilities. More than 10,000 cultivars are maintained in the CIAT germplasm bank alone.

These species are native to the new world, where they are commonly grown in association with maize and under rather infertile soil conditions. More detailed descriptions are given by Miranda (1966) and Purseglove (1968). In 1974 worldwide production of *P. vulgaris* totalled 11.458 million metric tons with 22.9% from Latin America. Yield average for the region was only 640 Kg/ha (FAO, 1974) Production and yield figures for the other species are not listed.

SPECIFICITY IN THE *RHIZOBIUM* - *PHASEOLUS* ASSOCIATION

Two groups of root nodule bacteria nodulate *Phaseolus* species. Fast growing rhizobia of the "*R. phaseoli*" type nodulate *P. vulgaris* and *P. coccineus* (Walker, 1928; Baldwin and Fred, 1929; Buchanan and Gibbons, 1974). The same authors report *P. angustifolia* also nodulated by *R. phaseoli*, but this species is not listed in even the older *Phaseolus* classifications (Hendrick *et al.*, 1931). *R. phaseoli* nodulates neither *P. lunatus* nor *P. acutifolius* and is non infective to the Asian species

previously included in this genus (Barua and Ghaduri, 1967).

Dart (1974) reports nodulation of *P. coccineus* by an ineffective strain of *R. leguminosarum;* otherwise this species appears specific in the rhizobia it will accept (Wilson, 1944). Some nodulation barriers may even exist between *P. vulgaris* and *P. coccineus* in that not all the *R. phaseoli* isolates we have tested nodulate the latter species. By contrast *P. vulgaris* is notably promiscuous, nodulating with isolates from *Amorpha, Anthyllus, Caragana, Crotalaria, Dalea, Desmodium, Onobrychis* and *Robinia* (Sears and Clark, 1930; Wilson, 1944; Gregory and Allen, 1953; Rangaswami and Oblisami, 1962), and with rhizobia from legumes indigenous to South-Western Australia (Lange, 1961). Though isolates from Asian species previously included in *Phaseolus* do not nodulate *P. vulgaris* (Barua and Bhaduri, 1967), we have obtained nodulation of *P. vulgaris, P. lunatus* and *P. acutifolius* with two slow-growing strains from *P. adenanthus*. Reconciling such promiscuity with the specific bean phytohaemagglutin -*R. phaseoli* reaction proposed by Hamblin and Kent (1973) is difficult.

Walker (1928) and Carroll (1934) place *P. lunatus* and *P. acutifolius* in the "cowpea" cross inoculation group, the former nodulating with isolates from cowpea, *Lespedeza* and *Stizolobium*. Rhizobia from *P. lunatus* nodulate *Canavalia, Clitoria, Lablab* and *Vigna* (Carroll, 1934). Within the cowpea group both species appear restricted in the number of strains with which they will form effective symbioses (Burton, 1952; Graham, unpublished).

Species differences in nodulation parallel hybridisation boundaries. Natural and artificial hybridisation between *P. vulgaris* and *P. coccineus* is common (especially with the former as the seed parent) (Al Yasiri and Coyne, 1966; Smartt, 1970), and several intermediate forms are known. *P. vulgaris x P. acutifolius* hybrids, though obtainable, are normally sterile (Honma, 1956; Coyne, 1964; Smartt, 1970) but *P. lunatus* does not cross with any of these species. *P. polyanthus, P. formosus* and *P. ritensis* appear intermediate between *P. lunatus* and the remaining species (Marechel, pers. comm.), but their *Rhizobium* affinities are not known.

NITROGEN FIXATION IN *PHASEOLUS* SPECIES

Phaseolus vulgaris has the dubious honor to be the first legume for which parasitic strains of *Rhizobium* were identified (Frank 1890, cit. Allen and Allen, 1958). Its subsequent inoculation history has been little better. Thus, though 65 - 70% of soybean seed planted in Brasil is inoculated, less than 0.5% of bean seed is so treated (Araujo, 1974). Bean inoculant production in the USA is similarly low (Burton, 1967). Reports that the percentage of nodule cells infected is lower in beans than in either soybean or peanut (Vincent, 1974) and that specific nodule activities in beans are less than half those commonly reported for soybeans (Hardy *et al.*, 1968) imply that *Phaseolus* is very much the poor relation among nodulated legumes.

respond to inoculation is common (El Beheidi, 1970; Fontes, 1972).

Brakel (1966) obtained yield increases of up to 98.6% with three of 6 strains of *R. phaseoli* tested. Growth was similar to plots receiving 50 units of N fertilizer / ha and uninoculated plots showed clear deficiency symptoms. In Madagascar, yields rose from 1272 to 2315 Kg/ha following inoculation, though the crop cycle lasted only 70 days (Vuong Hui Hai, 1970); while in the Sudan, Habish and Ishag (1974) report yield increase from 793 to 1943 Kg/ha in 1970/71 and 1512 to 1726 Kg/ha in 1971/72.

In our experience *P. vulgaris* responds well to inoculation, but within a relatively narrow environmental range. In one study where 10 varieties were compared, seed nitrogen gains from inoculation averaged almost 30 Kg/ha during the 120 days growth period. The strain used, CIAT-57 = CC511 was field tested in 1975 against 60 other isolates, but none showed superior fixation.

Though competition for nodule sites between inoculant strains and native soil rhizobia can occur (Ireland & Vincent, 1968; Law and Strijdom, 1974) it has not been reported as a problem in beans. In California it has been shown that soil rhizobial populations gradually increase following the introduction of lima beans to new ground even in the absence of inoculant, but take several years to cause significant nodulation (Allard, 1953). In inoculant trials with *P. vulgaris* the percentage of uninoculated plants with nodules has ranged from 4 to 28.7% (Norris *et al.*, 1970; Sistachs, 1970) while nodules per uninoculated plant varied from 0.08 to 38.0 (Norris *et al.*, 1970; Vuong Hui Hai, 1970). Burton *et al.*, (1952) found *R. phaseoli* not abundant in 10 mid-western soils.

Characteristics of Nodule Development and Fixation

Though Franco & Döbereiner (1967) earlier reported differences between varieties in nodule development and fixation, this field has been little studied. Recently we have emphasised varietal testing, field screening more than 150 varieties for their response to inoculation and for nitrogen fixation differences. Interesting varieties are identified in small scale plantings where nodulation, yield and N gains from inoculation (Fig. 3) are emphasised. Further testing uses field plantings (250,000 plants/ha) with weekly samplings for acetylene reduction.

Typical varietal profiles for acetylene reduction are shown in Fig 4, with nodule dry weight profiles very similar.

Acetylene reduction rates up to 38 µmol C_2H_4 produced/plant/hr have been recorded, and rates exceeding 20 µmol C_2H_4 produced/plant/hr are common. Specific nodule activities range from 130-250 µmol C_2H_4 produced/g dry weight nodule/hr. Such figures compare favourably with data reported in other species (Hardy *et al.*, 1968; Dart and Day, 1971; La Rue and Kurz, 1973 Lawrie and Wheeler, 1973; Dart *et al.*, 1976) and were similar to or greater than values achieved for soybeans grown under the same experimental conditions (see Fig. 5).

Greenhouse Evaluation of N_2 Fixation in Beans

Despite the depressing record cited above, *P. vulgaris* and *P. lunatus* respond satisfactorily to inoculation under screenhouse or greenhouse conditions (Burton *et al.*, 1954, 1961; Sandmann, 1970; Aguilera, 1974). Plant dry weight changes vary with strain and incubation period but reported increases range from 49 - 445%. In strain trials undertaken by Brakel (1966) plant nitrogen increased 225 - 539% in 60 days while Burton *et al.*, (1954) found total N increased from 15.4 to 49.3 mgm/plant.

The majority of legumes exhibit a distinct primary root around which the first nodules are clustered. By contrast *P. vulgaris* cultivars have a fibrous root system, with many small nodules located principally on secondary roots (Kamata, 1958) as shown in Fig. 2. There is a stronger tendency to tap root development in *P. coccineus*. Both species nodulate extremely rapidly under laboratory conditions with little strain or varietal difference apparent. Variety-strain combinations do differ, however, in nodule number / plant, as shown in Table 1.

Field Response to Inoculation

Field response to inoculation in beans is extremely variable, ranging from yield decreases when soil nitrogen was not limiting (Pessaha *et al.*, 1970; El Nadi, *et al.*, 1972) to substantial yield increases (Brakel, 1966; Pachecho Basurco, *et al.*, 1969; Vuong Hui Hai, 1970; Pillay and Mamet, 1972; Habish and Ishag, 1974). Failure to

TABLE 1. AVERAGE NODULE NUMBER PER PLANT*, SPECIES: PHASEOLUS VULGARIS

STRAIN	VARIETY									STRAIN MEAN
	CALIMA	LINEA 16	DUVA	LINEA 32	HUASANO	CARGAMANTO	BUNSI	TUI	GUALI	
114	152.1	32.2	80.1	39.7	28.8	69.6	25.8	33.7	66.3	58.7
57	47.0	93.4	41.3	32.3	41.3	62.9	15.7	22.0	54.9	46.6
129	48.6	60.9	207.6	31.2	31.2	66.0	10.9	34.0	92.3	65.0
95	18.7	34.4	48.6	24.2	11.1	56.8	29.2	51.7	78.8	39.2
VARIETAL MEAN	66.3	55.2	94.4	31.8	28.1	63.8	20.4	53.3	74.3	

* AVERAGE VALUE FOR 9 PLANTS
READINGS TAKEN AT 30 DAYS
F (VARIETY) 25.83** F (STRAIN) 13.26** F (VARIETY x STRAIN) 8.88**

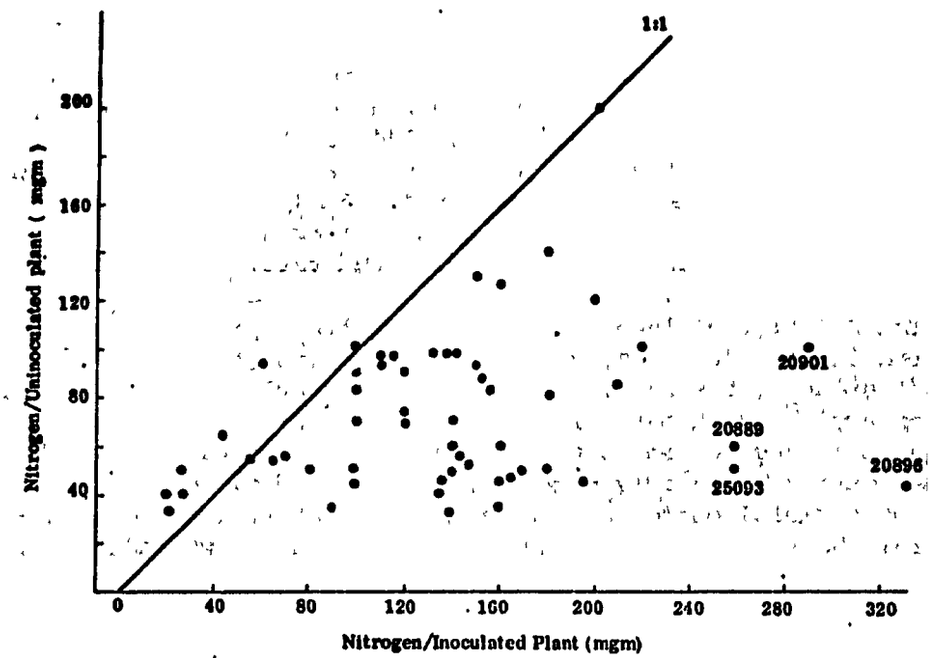

Fig. 3. Response to inoculation of 53 varieties of P. vulgaris. Varietal reaction is indicated by the total nitrogen/plant (mgm) of uninoculated vs. inoculated plants. Varieties below the line benefited from inoculation.

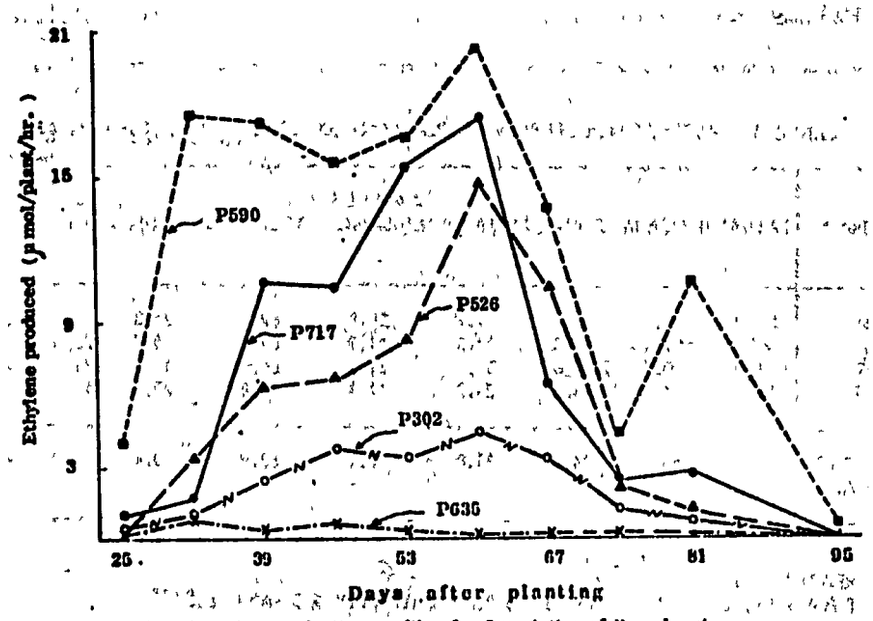

Fig. 4. Acetylene reduction profiles for 5 varieties of P. vulgaris.

In the only reasonably comparable field study located, Janssen & Vitosh, (1974) obtained fixation rates in beans up to 6 µmol C_2H_4 produced/plant/hr. Glasshouse data given by Sprent (1976) is equivalent in some experiments to only 1.5 µmol C_2H_4 produced/plant/hr.

For us, bush beans have proved weaker in nitrogen fixation than the botanically more primitive climbing cultivars (Fig.5, Graham and Rosas, in press). The cultivar P 590, outstanding in nitrogen fixation over several seasons, and possessing extremely high soluble and insoluble carbohydrate levels in the nodules (see below) is included here for comparison. In these experiments bush cultivars assimilated more combined N than climbers in the prefixation period (see Fig.6). This perhaps reflects unconcious selection for yield during cultivation of the plants under reasonable fertiliser conditions, and could in turn lead to depression in nodule activity (Small and Leonard, 1969).

The shortness of the fixation period in beans, evident in Figs. 4 & 5, has worried us. At 18-20°C few cultivars show activity until 4 weeks after planting and in most, activity begins to decline at about 10 weeks. This can be related in some degree to carbohydrate supply, as discussed in the next section, but the relationship is not a simple one. We do not believe currently that the potential exists in beans for a fixation period equivalent to those of soybean or peanut (Hardy *et al.*, 1971).

Carbohydrate Supply and Nitrogen Fixation

Though the energy requirement for fixation in beans is not known, two assumptions can be made based on other grain legume studies. (1) Carbon skeletons will be required in the nodule for transport of fixed nitrogen to the shoot. This is in addition to the normal respiratory requirement which though high is not appreciably greater in nodules than in some other plant parts (Allison *et al.*, 1940; Minchin and Pate, 1973). Thus, Minchin and Pate (1973) estimate that as much as 32% of the net carbon gain of pea plants is utilized in support of nodule function. (2) Nitrogen fixation will fluctuate diurnally and seasonally as the ability of the shoot to meet nodule demands changes. In cowpeas, for example, pool carbohydrate will support nodule function for only 1 - 2 hours in the absence of fresh inputs (Halliday, 1976).

We are beginning work to better understand how varietal differences in nitrogen fixation might relate to carbohydrate supply and availability in the bean nodule. In one experiment 14 commercial varieties, inoculated with CIAT strain 57 were sampled at day 39 (initiation of fixation) and 61 (beginning of decline in fixation rates) days after planting. All plant parts were sampled and analysed for ethanol soluble and insoluble carbohydrates. The acetylene reduction data of Fig. 4 were part of this study.

Marked varietal differences were found. In the early harvest soluble carbohydrate concentrations in the nodule ranged from

0.3 - 2.6% on a fresh weight basis. For roots, stems and leaves soluble carbohydrate levels varied from 0.7 - 1.8%, 0.4 - 1.4% and 0.6 - 2.7% respectively. Compounding these differences with variation in organ mass extended the range considerably while by the second harvest, differences were even more exaggerated.

Tables 2 and 3 compare the varieties P590 and P635; the former highly active in nitrogen fixation, the latter inactive despite copious nodulation (see Fig. 4). As shown in these tables, P 590 not only has a higher soluble carbohydrate percentage in all organs but also partitions more of its total carbohydrate to the nodule.

In this study there was a strong correlation between carbohydrate supply and acetylene reduction. Fig. 7 relates total soluble carbohydrate in the nodule in acetylene reduction, with the suggestion that nitrogenase activity could be saturated at high nodule carbohydrate content. In Fig. 8 a correlation between specific nodule activity and soluble carbohydrate in the nodule is evident at day 39, but does not exist at day 61. This could reflect the state of nodule development at these two periods. As shown in Fig. 9, major changes in distribution of carbohydrate between root and nodule are needed to boost nitrogen fixation levels during the period of active nodule development. At the later harvest, when nodule development is not important the nodule responds to small changes in available carbohydrate, but may be limited in activity by degeneration of the nodules.

The results might also explain the superiority of the climbing varieties in nitrogen fixation (Fig. 5). Of the additional varieties

TABLE 2 : DISTRIBUTION OF CARBOHYDRATE * (mgm/plant) IN TWO VARIETIES OF *P. VULGARIS* AT TWO STAGES IN PLANT DEVELOPMENT.

PLANT PART	DAY 39		DAY 61	
	P590	P635	P590	P635
NODULES	35.35	10.94	100.62	0.92
ROOTS	55.29	90.84	175.39	247.04
STEM				
nodes 1 + 2	124.58	395.13	110.07	678.68
nodes 3 + 4	118.68	118.67	414.51	71.97
nodes 5 + 6	52.23	-	111.06	174.23
nodes 7 + 8	-	-	157.44	-
> 8	-	-	70.40	-
LEAF				
nodes 1 + 2	270.02	336.44	191.50	88.03
nodes 3 + 4	114.48	171.38	404.65	60.29
nodes 5 + 6	66.40	-	116.60	99.06
nodes 7 + 8	-	-	227.43	-
> 8	-	-	51.50	-
PODS	-	-	-	132.76
TOTAL	837.03	1124.40	2121.17	1552.98

* both ethanol soluble and insoluble CHO

TABLE 3 : DISTRIBUTION OF CARBOHYDRATE (AS % OF TOTAL PLANT CHO) IN SELECTED VARIETIES OF P. VULGARIS AT 39 AND 61 DAYS AFTER PLANTING.

PLANT PART	CHO SOURCE	MEAN 14 VARIETIES 39	MEAN 14 VARIETIES 61	P 590 39	P 590 61	P635 39	P635 61
NODULES	SOLUBLE *	0.68	0.34	1.31	1.84	0.61	0.02
	INSOLUBLE	1.40	0.69	2.87	2.81	0.29	0.01
	TOTAL	2.08	1.03	4.21	4.65	0.90	0.06
ROOT	SOLUBLE	3.82	2.95	4.02	3.71	5.08	4.09
	INSOLUBLE	2.46	5.40	2.58	4.41	3.00	11.82
	TOTAL	6.28	8.35	6.60	8.12	8.08	15.91
STEM	SOLUBLE	10.92	16.52	15.90	28.46	15.76	5.29
	INSOLUBLE	17.66	25.22	19.39	11.19	29.98	54.26
	TOTAL	28.58	41.74	35.29	39.95	45.74	59.55
LEAVES	SOLUBLE	20.36	21.62	29.30	31.53	15.13	4.33
	INSOLUBLE	43.33	25.98	21.56	15.75	29.78	11.60
	TOTAL	63.69	47.60	53.86	47.28	45.21	15.93
PODS	SOLUBLE	-	0.90	-	-	-	5.17
	INSOLUBLE	-	0.78	-	-	-	3.38
	TOTAL	0	1.68	0	0	0	8.55
TOTAL	SOLUBLE	35.25	42.33	50.59	65.56	36.50	18.90
	INSOLUBLE	64.78	58.07	49.41	34.44	63.50	81.10

* ethanol soluble

tested here the best five in acetylene reduction were all climbers, and, as shown in Fig. 10, tended to hold more of their carbohydrate in the soluble form. The only climbing variety with soluble carbohydrate less than 30% of the total proved poor in acetylene reduction. The carbohydrate content of the nodule or root is only a small fraction of their through-put. Although through-put cannot be measured in the absence of translocation rate data, there is certainly strong presumptive evidence to relate a plant's soluble nitrogen content to the amount which can be sequestered by the nodules.

Influence of Soil Temperature on Nodulation and Fixation

Temperature is a major factor limiting fixation in beans in tropical and subtropical areas. Our two work sites, separated by only 140 Km are at altitudes of 1000 and 1800 m respectively. At the higher elevation with a maximum soil temperature (10cm) of 29°C, fixation rates commonly exceed 20 µmol C_2H_4 produced/plant/hr. At the lower site, where soil temperatures (10cm) can exceed 35°C for several hours a day, the maximum fixation rate recorded is less than 10 µmol C_2H_4 produced/plant/hr. The effect is principally on rate of fixation, though high temperatures can also cause poor nodulation in *P vulgaris*. (Barrios, *et al.*, 1963; Small *et al.*, 1968; Graham and Hubbell, 1975; Halliday and Pate, 1976).

Barrios *et al.*, (1963) used the isolated root technique, to compare temperature effects on nodulation. Greatest nodule number/root and % root nodulated were obtained at 25°C, but no nodules were formed at 33°C.

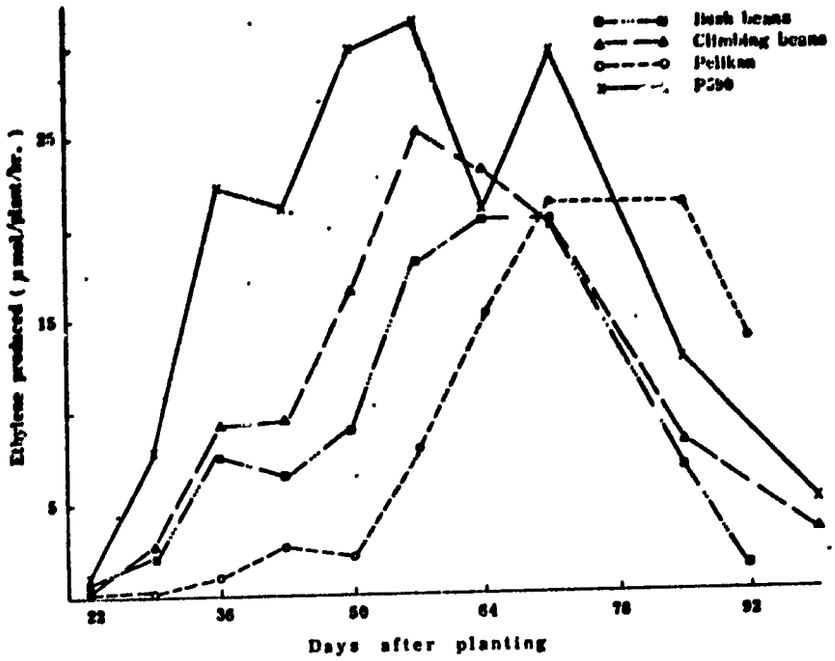

Fig. 5. Acetylene reduction profiles for bush and climbing cultivars of P. vulgaris. Each data point averages results for 10 varieties. The cultivar P590 and the soybean variety Pelikan are included as checks. (Graham and Rosas, in press).

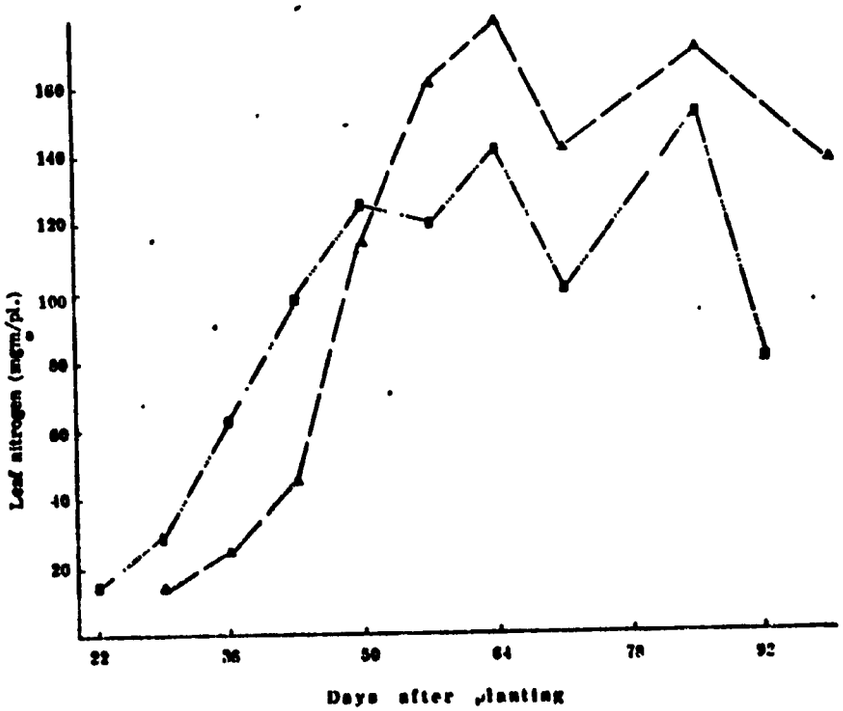

Fig. 6. Nitrogen content of leaves of bush and climbing cultivars of P. vulgaris measured at different stages of the growing season.
Each data point averages 10 varieties each of 4 replications (Graham and Rosas, in press)

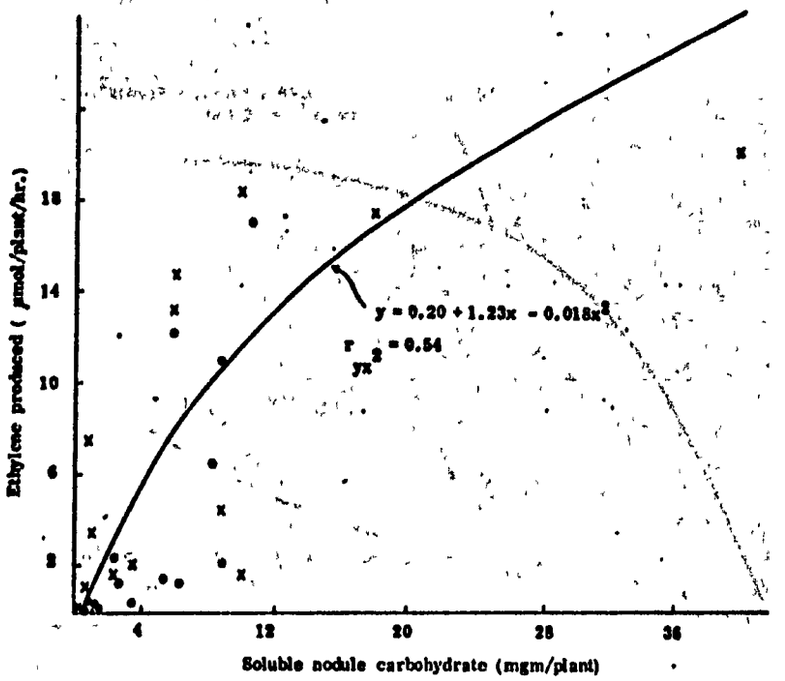

Fig. 7. Relation between soluble nodule carbohydrate and acetylene reduction levels in 14 varieties of P. vulgaris at 39 (•) and 61 (x) days after planting.

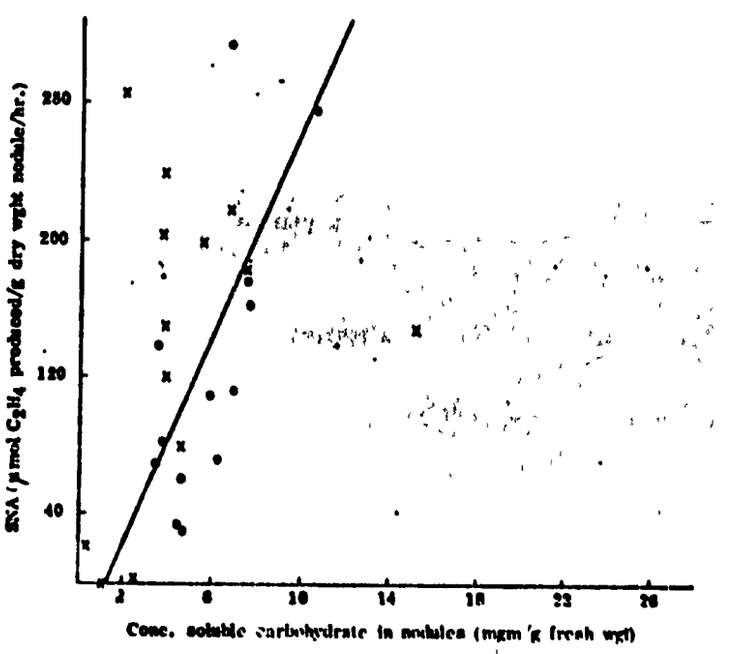

Fig. 8. Relation between CHO concentration in nodules and specific nodule activity in 14 varieties of P. vulgaris sampled at 39 (•) and 61 (x) days after planting. For the earlier harvest and for all varieties save P589
y = 28.11x- 32.50, r = 0.659

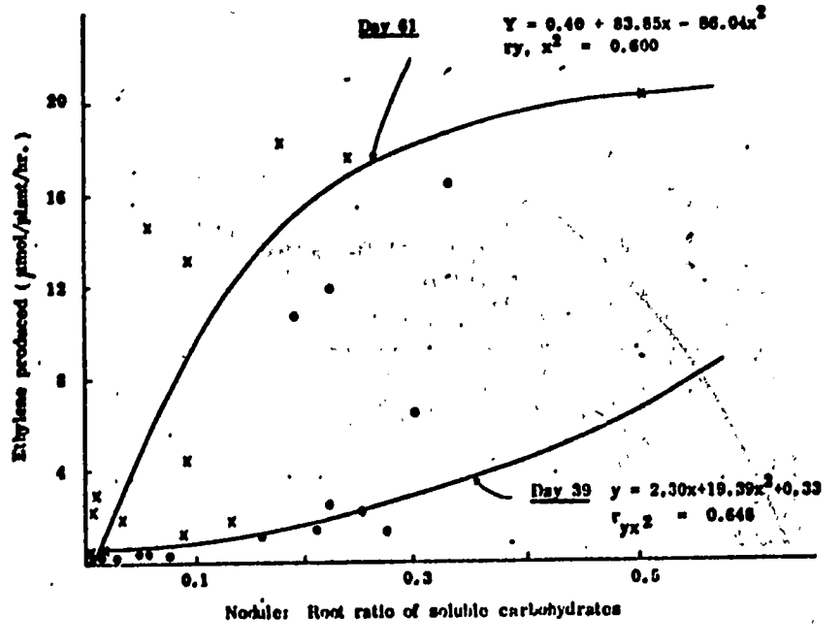

Fig. 9. Relation between acetylene reduction levels and the distribution of ethanol soluble carbohydrate between nodules and roots of 14 P. vulgaris varieties x 61 day • 39 days after planting

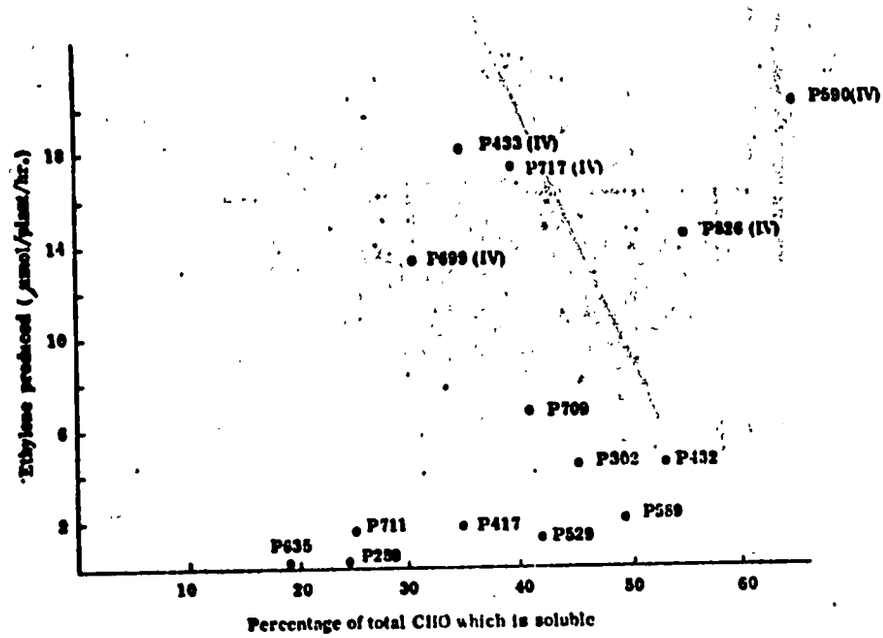

Fig. 10. Relation between acetylene reduction in beans and the percentage of carbohydrate which is ethanol soluble. 61 days after planting. The subscript (IV) identifies climbing varieties.

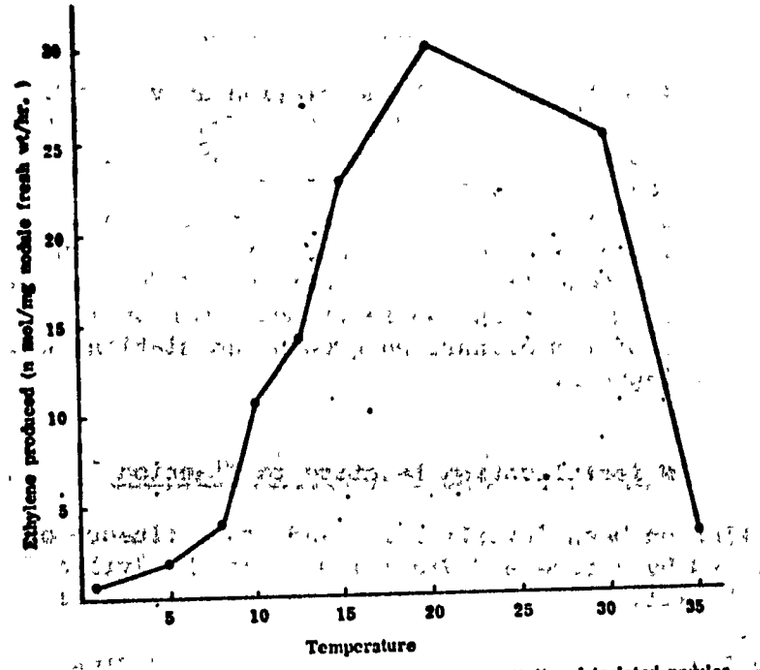

Fig. 11. Effect of temperature on nitrogenase activity of isolated nodules of P. vulgaris. Each point is the mean of 4 replicates; standard errors 0.43-3.90 n moles
Source: Pankhurst and Sprent, 1976.

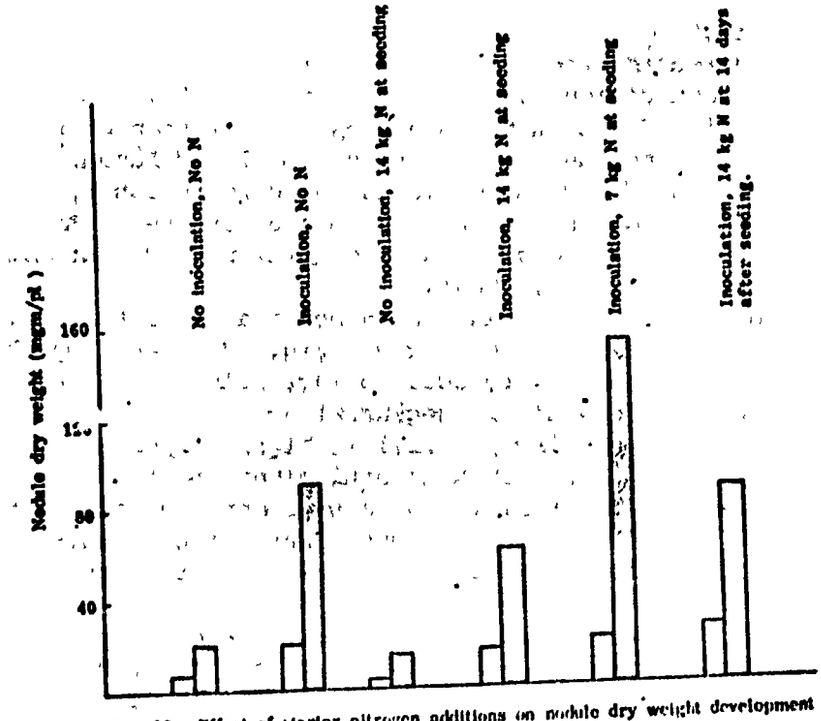

Fig. 12. Effect of starter nitrogen additions on nodule dry weight development at Popayán.

Similarly Lie (1964) obtained little nodulation above 30°C. Fig. 11 shows fixation rates in detached nodules as a function of temperature. Optimum fixation occurred over the range 15 - 30°C with a decline in fixation of more than 80% between 30 and 35°C. Halliday and Pate, (1976) provide similar data for clover. Varietal responses to temperature do differ. Day (pers. comm.) compared fixation rates in 6 cultivars over the range 18 - 36°C. None fixed at a continuous 36°C the lines 26689 and 20972 were more active than the remainder. Gibson (1971) has reviewed the effect of temperature on growth, nodulation, and nitrogen fixation in other legumes.

N Fertilization Relative to Fixation

Information on bean fertilization and its influence on fixation has been reviewed by Friere and Vidor (1970) and by Avilio Franco in these proceedings. We will limit our comments to the effects of nitrogen fertilization.

High soil nitrogen or nitrogen fertilization decreases or delays nodulation (Cartwright, 1967; Sistachs, 1970; El Nadi *et al.*, 1972) and limits the contribution of fixed nitrogen to overall plant development. Additions in the range of 5 - 15 Kg/ha can, however, promote nodule development. (Fig. 12).

In the root zone, combined nitrogen acts principally against nodulation, decreasing the production of indolyl acetic acid (IAA) or hastening its destruction (Tanner and Anderson 1964, 1965). Within the plant high levels of nitrogen cause greater retention of photosynthate in the root, limiting the nodule supply (Small and Leonard, 1969). Nitrate inhibition of nodulation in isolated roots can be prevented by increasing sucrose concentrations fed to the cut stem. At higher carbohydrate levels, nitrate in the stem vial promotes nodulation (Raggio, Raggio and Torrey, 1965).

Under our conditions fixation in beans appears to contribute less than 50% of total plant nitrogen. Fig. 13 shows N uptake in the variety Porrillo sintético, to occur principally in the period after flowering when nodules would be degenerating. As shown in this figure, leaf nitrogen decline during pod fill is highly important in tropical areas. Even at 60 Kg combined N/ha equivalent, potted plants receive almost two thirds of their nitrogen requirement from fixation (Dart *et al.*, 1976). Spaced glasshouse plants might be expected to photosynthesise more or to maintain leaf acitivity longer. Such results therefore require careful interpretation.

Given the decline in fixation and the increased nitrogen demand for bean development in the post flowering period, we have attempted late applications of fertilizer N. Maximum yields were obtained in nodulated plants to which supplemental N fertilizer was applied 50 days after germination.

Soil Acidity

The limiting pH for the growth of bean rhizobia in liquid medium is from 4.0-4.4 (Ishizawa 1953; Graham and Parker, 1964). In soil, acidity is often coupled to aluminum or manganese toxicities (Döbereiner, 1966; Souto and Döbereiner, 1969; Morales, Graham and Cavallo, 1973), with nodulation problems in beans increasingly common as the soil pH drops below pH 5.2. Though Nutman (1972) considers nodule formation more sensitive to low pH than are nodule bacteria alone, Danso (cited Graham and Hubbell, 1974) observed a 99% decline in viable cells of *R. phaseoli* during 14 days in an acid soil.

Soil liming is a common practise in Latin America but can have deleterious effects on phosphate availability (Pearson, 1975) or prove uneconomical. Seed pelleting of beans has also been widely practised (Pacheco Basurco *et al.*, 1969; Norris *et al.*, 1970; Morales *et al.*, 1973); ground rock phosphate being substituted for limestone with acceptable results (Ruschel, *et al.*, 1970; Morales *et al.*, 1973). Damage to the integrity of the pellet caused by imbibition and seed swelling is a common problem with beans.

Ruschel *et al.*, (1970) incorporated boron and molybdenum in the lime used for pelleting and obtained yield and nodule weight increases. This practice is risky especially when sodium molybdate is used as Mo source. (Burton and Curley, 1966; Graham, Morales and Zambrano, 1974).

Though *P. vulgaris* is less well adapted to acid soils than is *P. lunatus*, some black-seeded cultivars show reasonable acid tolerance (Spain *et al.*, 1974). The *Rhizobium* relationships of such cultivars have still to be studied.

CONCLUSIONS

There are many aspects of the *Phaseolus* -*Rhizobium* symbiosis which still require investigation. Not all relate to the agricultural aspects of the symbiosis.

Definition of differences in cross -inoculation between *P. vulgaris* or *P. coccineus* and *P. lunatus* and of the requirements for nodulation in lesser *Phaseolus* species is needed. Inheritance of root and nodule characteristics in *P. vulgaris* x *coccineus* hybrids could also be studied.

Spain *et al.*, (1974) identified cultivars of *P. vulgaris* tolerant of acid soil conditions. Studies of the infection process and nodule formation in these cultivars could promote knowledge of the "acid - sensitive" steps in legume nodulation (Munns, 1968; Lie, 1969). Lie (1971) obtained an isolate of *R. leguminosarum* tolerant of acid soil pH but moderately efficient in fixation. Similar strains of *R. phaseoli* could exist, and in combination with selected cultivars prove more durable under acid soil conditions.

Though much bean production in Latin America occurs outside the tropics or in high mountain valleys, bean areas in Central America, Brasil, Colombia and Venezuela experience soil temperatures above

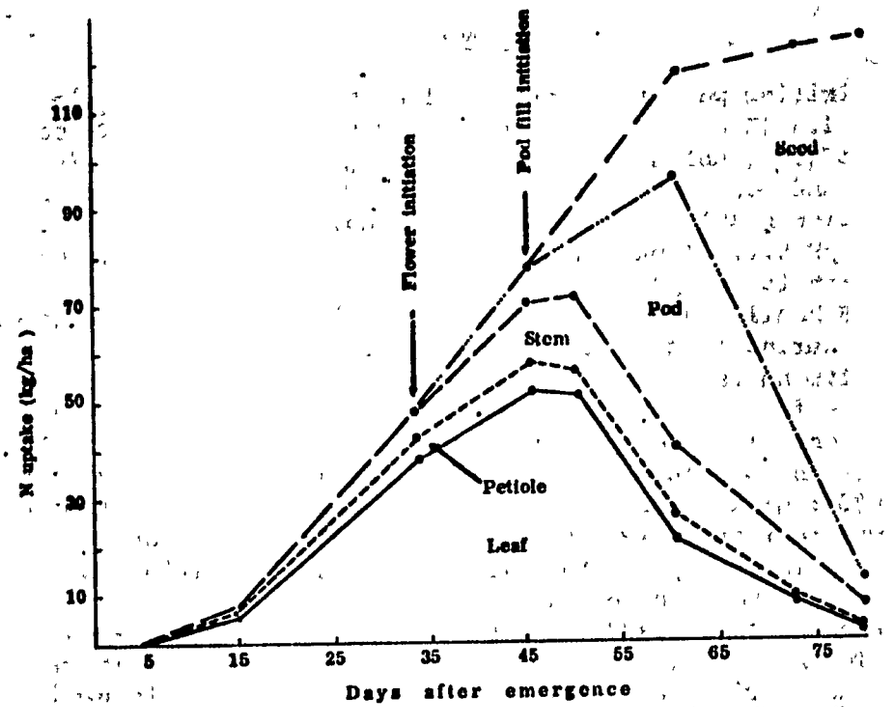

Fig. 13. Uptake of nitrogen by the variety Porrillo Sintético during its growth cycle. Note the large percentage of N absorbed after flowering, and N loss from leaves and pod wall during grain filling. (After Ceballos, Laing and Fernandes, in press).

those optimal for nitrogen fixation. Though organic matter incorporation sometimes increases yield (Guazzelli and Miyasaka, 1971) and/or nodule formation (Norris *et al.*, 1970), it is often not practical as a remedy. Associations of maize and beans after an alternative solution, the maize both supporting the bean and limiting soil temperature and desiccation. Competition from the maize for light and nutrients could limit bean fixation, but as the bean canopy saturates at the relatively low light intensity of 600 Wm^{-2} (Sale, 1975) it is better suited than most to achieve action fixation under shaded conditions. Further studies are needed.

Temperature adaptation in *Rhizobium* strains has been demonstrated (Ek Janders and Fahraeus, 1971; Gibson, 1971; Lie, 1971). Thus strains of *R. trifolii* isolates in Scandinavia grew faster, nodulated more quickly and fixed more nitrogen at 10°C than did isolates from warmer areas (Ek Janders and Fahraeus, 1971). With the Talarook cultivar of *T. subterraneum* the strain TA I fixed almost as well at 30°C as at 22°C while strain NA 30 was only active at the lower temperature (Gibson, 1971). While these studies emphasise the rhizobial component of the symbiosis the host offers similar opportunities for selection

Though the role of the host plant in providing energy for nodule development and fixation has long been recognized, the introduction of the acetylene reduction technique (Hardy *et al.*, 1968) has reawakened interest in the energy relationship between plant and microbe. Plants have been shaded (Lawrie and Wheeler, 1973; Chu and Robertson, 1974;

Lawn and Brun, 1974a; Sprent, 1976; Halliday and Pate, 1976), defoliated (Chu and Robertson, 1974; Lawn and Brun, 1974g; Halliday and Pate, 1976), depodded or deflowered (Lawn and Brun, 1974a; Lawrie and Wheeler, 1974), given supplemental lighting (Lawn and Brun, 1974a), spaced further apart (Sprent, 1976), grafted (Streeter, 1974; Lawn and Brun, 1974b) and supplied additional CO_2 (Hardy and Havalka, 1976).

In this last experiment, total fixation in CO_2 supplemented plants was almost five-fold that of normal soybeans. Energy balance sheets for the plant and nodule have been calculated (Minchin and Pate, 1973), but as a generality those treatments which enhance photosynthesis, or eliminate alternate energy sinks, enhance fixation, and vice versa.

None of the investigations cited have used *Phaseolus vulgaris*. This is unfortunate, since with this species there is a high probability that such information would find a practical outlet. Several aspects of our current experimentation, most stressing varietal selection, could illustrate this point.

1. In beans, as in soybeans, varieties differ in flowering and maturity characteristics. At our Popayan site different germplasm accessions flower in from 32 - 68 days, and mature in 90 - 130. As Hardy *et al.*, (1971) reported, and we can confirm, lateness and higher nitrogen fixation are associated. Where rainfall or temperature limitations permit, selection of later maturing varieties is likely to extend the fixation period and increase total N_2 fixed.
2. While flowers and pods provide alternative sinks for carbohydrates, and compete with fixation (Lawrie and Wheeler, 1974) lower leaf life also appears involved in the decay of nodule activity. When $^{14}CO_2$ is fed to leaves 1 and 2 of indeterminate bean varieties, most activity is recovered in the lower stem or roots. Both root and upper stem nodes benefit from feeding at leaf 3, but feeding at leaf 6 has virtually no effect on root photosynthate (Tanaka, pers. comm.). Leaf loss in beans under tropical conditions is pronounced (Table 2, Fig. 13) the lower leaves being most affected. By selecting varieties with a canopy structure permitting longer life in the lower leaves, it is hoped also to improve nitrogen fixation.
3. Differences between varieties in nitrogen fixing ability can be related to carbohydrate supply and form. Holl and La Rue (in press) show similar variation in *Pisum*, reporting a gene "sym 3" which controls the amount and/or the form of carbohydrate supplied to nodules. Further varietal studies are needed in beans. CIAT with its large germplasm bank is well placed to select for desired features. Some interesting materials, particularly P 590 and P 635, have been mentioned already.

If the ability to fix appreciable levels of nitrogen is to be introduced into commercial bean cultivars, breeding for a combination of yield and nitrogen fixation will probably be necessary. Genetic studies undertaken to date, have not really faced up to the problem,

most examining characters such as the loss of nodule forming ability (Nutman, 1949; Williams and Lynch, 1954; Lie, 1971) or lesions in nodule or bacteroid function (Nutman, 1954; Holl, 1975). Almost certainly efficiency in fixation will be quantitatively inherited, so that observations of nodules (Imrie, 1975), or of fixation at a single time in plant development, will fail to assay accurately the duration and intensity of the fixation reaction or the factors limiting it. Methodologies are needed for the determination of such quantitative differences, as are studies on the inheritance of effective symbiosis itself.

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INOCULATION OF SOYBEANS

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INTRODUCTION

Soybean *(Glycine max* (L.) Merril) production is expanding rapidly throughout the world as a result of the high demand for the grain as an excellent source of oil and protein. For a long period the United States and mainland China were the largest producers, far ahead of the other countries. However, about 15 years ago soybean production in Brasil started to grow rapidly and after the spectacular price increases in 1973, planting and production grew even faster. In 20 years (1955-1975), the area planted to soybeans has expanded from 80,000 ha to 8 million ha. In 1972 it was anticipated that the area of 8 million ha would be reached only in 1977 or 1978. Considering a conservative average of 70 kg of nitrogen fixed per ha by the symbiosis, we would have a saving of 560,000 tons of nitrogen taken from the air instead of from the soil or fertilizer. However, the interaction of several factors will control the actual N_2 fixed.

Success in obtaining high N_2 fixation through the symbiosis of *Rhizobium japonicum* with soybeans depends on a series of factors which sometimes interact increasing the complexity of the system:
1. effectiveness and efficiency of the *R. japonicum* strains present in the inoculum and/or soil in relation to the varieties and the environment; 2. number of rhizobia cells in the inoculum in relation to the native or naturalized population of *R. japonicum;* 3. techniques of inoculation and seeding to provide adequate survival and multiplication of rhizobia around the roots; 4. environmental factors, mainly limiting soil factors, which will affect survival of the introduced rhizobia in soil, their establishment, rapid nodule initiation and/or functioning, and the physiology of the plants. Therefore the effects are direct on rhizobia and indirect through the host plant. Fortunately and evidently most of the conditions that benefit the bacteria and N_2 fixation are also of benefit to the physiology, including nutrition, of the plant, and once these conditions are satisfied both partners are benefited and productivity will be high.

STRAIN SELECTION

The work of collecting, isolating and selecting effective, efficient strains of *Rhizobium japonicum* is usually fruitful, because variation between strains is large and most of them do not reveal the desired properties. Many institutions throughout the world supply cultures upon request, usually free of charge. Techniques of isolating, testing and maintaining cultures have been well treated by Vincent (1970, 1971), Roughley (1970), Norris (1964b) and other authors.

Cultures of *Rhizobium japonicum* supplied by the several agencies have certainly been subjected to efficiency selections and posses some of the desirable properties. However, the large number of local soybean varieties used for commercial plantings and the wide range in environmental conditions make local testing and selection advisable. It is also advisable to compare imported cultures with native isolates from old cropping regions where the bacteria are native or naturalized for a long time. Frequently these native isolates have adapted to the local environment and show higher efficiency, greater competitive ability, more tolerance to stress conditions and possibly other useful properties which would make these strains of value as inoculants. One feasible method of testing is to run the preliminary screenings in Leonard jars (with a large number of strains) with one or two soybean varieties. Smaller numbers of selected strains will then be tested against the other commercial varieties in the Leonard jars or directly in soil pots. One problem that may frequently arise when testing in soil is a high level of nitrates particularly when the soil is limed. Due to this problem we routinely use two soybean plantings, inoculating only the first and taking the results from the second. The differences between strains are then more marked. Further tests with strains may include comparisons between single strains and 2 x 2 combinations checking N_2 fixation during plant development and also testing for competitiveness for nodule sites and fixation by a mixture of strains with commercial soybean varieties. These second-step tests have been performed in the greenhouse, in pots with several soil types limed and unlimed. The last screening is performed in the field, in rhizobia-free soil and in as many locations and different environmental conditions as possible. These field experiments are expensive and difficult to conduct because of contamination between plots and also because of the difficulty in finding areas free of *R. japonicum*. Serological identification is needed for long-term evaluation and for checking contamination and survival in soil, etc. However when strains under test belong to the same serological group, identification is difficult or impossible. Routinely, the preliminary evaluation of strain performance is based on tissue dry matter and dry weight of nodules. In special or second-step tests total nitrogen or acetylene reduction assay may be used. Some desirable characteristics for selecting strains in tests involving Leonard jars and soil pots are: earliness and position of nodule formation at normal and at high temperatures, earliness and lateness of N_2 fixation. Tolerance to low

pH and toxic aluminum and manganese is also desirable to test for in order to have strains for use in areas of low input agriculture.

NODULATION

Root exudates (Vest, *et al.*, 1973) by the host, by non-host plants and even by non-legumes play an important role in the stimulation of *R. japonicum*. Gramineous plants do not seem to have this stimulation power. Equal numbers of *R. japonicum* were found in the rhizosphere of two near isogenic lines of soybeans, one nodulating and other non-nodulating. This stimulation is important for nodulation as nodule number is positively related to bacterial density in the vicinity roots. One nodule is usually originated by the penetration of one rhizobia cell; however cells from two strains may penetrate and contribute to formation of the nodule. The occurrence of double-strain nodules depends on the relative densities of the two populations. Means, *et al.*, (1961) have found that, with rare exceptions a single nodule contained only one bacterial strain. Lindemann, *et al.*, (1974) used the fluorescent antibody technique and Leonard jars to test competition for nodule formation. After inoculating with 10^8 cells of two strains per plant, they found that 32% of the nodules contained both strains and that the percentage of double-strain nodules decreased with the decreasing density of the inoculum. At equal inoculation rates one strain would be more efficient in the formation of single-strain nodules, and the strains which formed more single nodules also predominated in the number of cells inside double-strain nodules.

The longevity of a single nodule in the field is still a matter of doubt. In greenhouse tests, Bergersen (Vest *et al.*, 1973) established an active life of 6-7 weeks. In the field, nodules appear 6 days after planting and N_2 fixation starts 2-3 weeks later, being really active from 3-4 weeks after planting to near maturity 12-13 weeks later. This period is probably the sum of the functional periods of a series of nodules which start appearing at the tap root and which are the first to decay.

Attempts to describe nodule characteristics and specific interactions by correlation of nodulation and fixation characteristics with plant properties have involved determination of the number, size, weight and color of nodules and nitrogen contents of the plant parts (Vest *et al.*, 1973). The terms effective and ineffective are inadequate to describe the many phenotypical nodulation responses, according to the above mentioned authors, and they have proposed a more precise classification, based on a clearer distinction between the terms ineffective, effective, inefficient and efficient. *R. japonicum* strains differ in effectiveness or the ability to produce nodules and also differ in efficiency or ability to fix atmospheric N_2. They have been divided into two categories: A. those which produce clusters of nodules on the tap root; B. those which produce scattered nodules on the lateral roots. Type A is reported to fix significantly more N_2 than type B and in the evaluation of strains and sufficiency of

nodulation, tap root nodulation is a valuable criterion. Weaver and Frederick (1972) and Burton (1975) also indicated that tap root nodulation is a valuable qualitative characteristic to determine adequacy of inoculation, population of rhizobia and sufficiency of nodulation. Rios and Döbereiner (1972), reported the following conclusions from a field experiment: 1. there was a significant varietal effect on total and mean nodule weight 2. there was an inverse relation between size and number of nodules 3. maximum number of nodules was reached before maximum weight.

Freire and Vidor (1974) have proposed an extensive classification of the types of responses to evaluate the efficiency of nodulation in the field and in experiments. This evaluation is based on the development of the plants, color of the leaves, and on the abundance, size, location, internal color and surface appearance of the nodules.

ECOLOGY OF *R. JAPONICUM* AND HOST VS STRAIN SPECIFICITY

Nodulation and N_2 fixation by the soybean vs *Rhizobium japonicum* symbiosis depend on a complex relationship between the two partners. One specific strain that nodulates and fixes N_2 on some varieties might fail with a genotype that is effectively nodulated by another strain. Some soybean varieties are more promiscuous, nodulating and fixing N_2 with a great number of strains. On the other hand, other varieties may be difficult to nodulate and only a few strains may be able to establish an effective and efficient symbiosis. Since the pioneer work of Williams and Lynch, several genes which control nodulation of soybeans have been identified. Vidor *et al.*, (1972), in a field experiment with 12 varieties, found significant interaction for phosphorus vs variety for nodule weight. Some of the easily nodulated varieties had twice the nodule weight of the poorly nodulated ones. In a serological identification of the nodule population of 8 varieties inoculated with a 3-strain, mixed inoculant, Araujo *et al.*, (1970) found high specific affinities between strains and varieties.

Plant genotypes exert a selective influence on strain populations. Caldwell and Vest (1968) found a significant difference among 16 plant genotypes in their *R. japonicum* acceptance. These 16 varieties were growing side by side in different fields in successive years. Closely related soybean genotypes had similar distributions of *R. japonicum* in their nodules. Results have indicated specific interactions between genotypes and rhizobia serogroups in nodule formation. A study by Erdman presented evidence that varieties adapted to a given area were better inoculated by the native strain than a variety introduced to the area. He also referred to the work of Johnson and Means who found that different *R. japonicum* populations were associated with different soils and that these populations remained quite constant over successive years. Skrdleta (1973b) however, observed no marked cultivar selections from different serogroups whether the inoculant contained 2 or 3 strains. One strain formed the greater proportion of the serotyped nodules on all the cultivars.

The effect of temperature on the recovery of *R. japonicum* serogroups was investigated by Weber and Miller (1972). They found that one serogroup which was dominant at a 30°C soil temperature formed fewer nodules at a low temperature and the inverse occurred with the other serogroup studied. In an experiment with 28 strains, 5 varieties and 3 locations, two being free of *R. japonicum,* Caldwell and Vest (1970) reached the following conclusions: 1. according also to other workers, there was a significant strain vs year interactions; 2. there was no significant interaction of strain vs variety; 3. the effect of the strains was influenced by environment; 4. in areas already populated by *R. japonicum,* the introduced strains produced only 5-10% of the nodules. And according to the review of de Mooy *et al.,* (1973) the serological distribution of nodules has been shown to vary considerably with location, genotype, planting dates and stage of plant development.

Most of the earlier ecological studies with *R. japonicum* were indirect, using the host plant. This approach, however, exerted selection pressure on the screening because the presence of the host plant favored development of certain strains among the soil population. Vest *et al.,* (1973) have reviewed the work done in this area. Schmidt, and Schmidt and Bohlool, using the fluorescent antibodies technique, have contributed to better understanding of the saprophytic stage of *R. japonicum* in soil. Also the use of serological identification has helped in studying the competition between inoculant and soil strains and the persistence in soil free of rhizobia. Several studies have indicated that the occurrence and persistence of *R. japonicum* are influenced by many factors such as planting date, soil type, percentage of sand and clay, temperature, moisture, organic matter, pH, antagonistic and beneficial microflora, other *R. japonicum* strains, etc. The effects of these complex factors, isolated or interacting, and the inherited properties of the strains determine the behavior of rhizobia strains in soil. Strains of *R. japonicum* differ in their ability to compete in soil free of native or naturalized strains. If one strain is applied at the time of sowing and other strain afterwards, the percentage of nodules produced by the one applied later decreases rapidly with time from sowing. In a field study now in the third year (Freire *et al.,* 1975 and unpublished results) seven strains of *R. japonicum* were inoculated only at first planting in individual plots in a rhizobia-free soil. One of the strains (587) is a stronger competitor and is gradually invading the neighboring plots, competing for and usually dominating the nodulation sites against the less competitive but also efficient strains inoculated in the plot and against the strains from the other neighboring plots. The relative predominance of the strains has varied with the year, but recovery of strain 587 has increased in almost all plots. In another experiment including 12 varieties inoculated with a four-strain inoculant, strain 587 predominated in 9 of the tested varieties. In several other experiments this strain dominated the nodulation sites in several types of soil. Another strain that had almost disappeared the first two years is now forming one third or more of the nodules of some varieties. In spite of the selective variety effect it appears that the recovery of a strain is based more

on its capacity for survival and competition for nodule sites than on the time from sowing and inoculation. Skrdleta (1970) also found that the number of nodules formed by a strain applied later and competing with the strain applied at sowing decreased rapidly with the time from sowing to inoculation. One strain was confirmed to be an especially strong competitor. In a study by Miller referred to by Vest, *et al.*, (1973) however the recovery of the applied strain was higher 60 days after planing than at an earlier stage. Another study on the impact of the first introduction indicated that a weak competitor is dominated when applied in an inoculum mixture, but when applied singly it can establish and even predominate in the soil. Another finding from a serological study is that distribution also differs when nodules are sampled at different stages of plant development.

Weaver *et al.*, (1972) found that a variation in pH from 5.0 to 8.0 did not affect the overall population of *R. japonicum*, but that individual strains may have a competitive advantage at a particular pH. These results were obtained from study of 52 fields in which *R. japonicum* populations ranged from 10 cells to more than 1 million cells of *R. japonicum* per gram. Numbers of *R. japonicum* in the rhizosphere were not significantly correlated with soil texture, soil pH, soil organic matter or presence of soybeans at sampling. Smith and Miller (1974) indicated a considerable influence of the interaction rhizobia vs indigenous soil microorganisms, on the ability of *R. japonicum* to become established in the soil. Kowalski *et al.*, (1974) found that distribution of phages in soil and nodules was related to the occurrence of *R. japonicum* serogroups in the nodules. They found phages and *R. japonicum* to be related to soil pH. Kuster (1974) found a close affinity of strains and varieties and that this affinity was changed by soil type. Through a change in the environment by liming, Kornelius, *et al.*, (1972) were able to introduce efficient rhizobia strains of *Trifolium subterraneum* in a soil dominated by a parasitic strain. This strain is efficient on *T. polymorphum* which is native in an extensive area of natural pastures of southern Brasil and Uruguay.

Some strains show much better performance in soil than others, but our knowledge is limited as to why this happens. An introduced strain has to defy the existing microflora, fight for the available substrate for growth and survival and compete for the nodule sites when the specific host is planted. According to Ham (1975) and Vest, *et al.*, (1973), who quoted H.W. Johnson, unless a way is found to enhance the competitive ability of more efficient applied strains, the search for more effective strain vs variety combinations and selection of better soybean varieties can be hampered because their performance can be affected by the naturalized *R. japonicum* populations in soil. They also point out the problem of selecting plant genotypes on the basis of their effective symbiosis in one location only to have them fail when tested in other areas where the *R. japonicum* population is different. The naturalized population of *R. japonicum* in breeding nurseries is unknown in many cases.

Table 1. Recovery of strains of *R. japonicum* inoculated at individual plots on 2 soybean varieties. Guaiba, RS - Brasil - 1976

Strains inoculated		Recovery (%) from varieties*						
		509	527	531	532	566	586	587
509	Bragg	-	2	1	-	21	16	56
	S.Rosa	-	-	2	-	16	36	45
527	Bragg	-	20	6	5	17	15	35
	S.Rosa	-	21	-	-	8	3	58
531	Bragg	-	-	1	-	17	40	35
	S.Rosa		9	4	1	22	3	61
532	Bragg	-	-	11	-	22	19	45
	S.Rosa	-	5	9	-	20	-	56
566	Bragg	-	9	2	1	16	12	55
	S.Rosa	-	1	3	4	20	11	56
586	Bragg	-	-	5	-	9	66	20
	S.Rosa	-	-	3	-	31	42	22
587	Bragg	-	-	9	-	6	15	68
	S.Rosa	-	4	-	-	11	15	68
Control	Bragg	-	-	11	1	17	22	47
	S.Rosa	-	3	6	3	32	2	50

* Cross reactions or nodules not identified are not indicated.

Table 2. Recovery of strains (%) of *R. japonicum* on a 3 year experiment with variety Bragg, Guaiba, RS - Brasil

Strain Inoc. 1973	Strain recovery*				Strain Inoc.	Strain recovery			
	Strain	1st y	2nd y	3 d y		Strain	1st y	2nd y	3 d y
	509	30	15	-		509	-	1	-
	509/32**	22	2	-		509/32	-	1	-
	527	-	-	2		527	5	-	8
509	531	-	-	1	566	531	-	-	2
	532	22	17	-		532	-	-	1
	566	-	-	21		566	2	-	16
	586	10	18	16		586	10	7	12
	587	15	37	56		587	82	90	55
	509	-	-	-		509	-	5	
	509/32	-	-	-		509/32	-	-	
	527	95	90	20		527	-	-	
527	531	-	-	6	586	531	-	-	5
	532	-	-	5		532	-	4	-
	566	-	-	17		566	-	-	9
	586	-	-	15		586	95	74	66
	587	5	10	35		587	5	16	20
	509	2	17	-		509	2	-	-
	509/32	77	16	-		509/32	-	-	-
	527	2	-	-		527	-	-	-
531	531	-	-	-	587	531	-	-	9
	532	15	16	-		532	-	-	-
	566	-	-	17		566	2	-	6
	586	-	16	40		586	-	-	15
	587	2	-	35		587	95	100	68
	509	15	7	-		509	11	5	-
	509/32	57	19	-		509/32	-	4	-
	527	-	1	-		527	5	1	-
	531	-	-	11		531	-	-	11
532	532	25	42	-	Control	532	37	6	1
	566	-	-	22		566	5	-	17
	586	-	15	18		586	5	30	22
	587	2	15	45		587	32	50	47

* Strain recovered from direct inoculation and contamination from other plots. Nodules not identified are omitted/

** Cross reactions.

Table 3. Recovery of 4 strains of *R. japonicum* of a mixed inoculant by serological identification from nodules of soybean varieties in 1975 and 1976 plantings of a field experiment. Guaiba, RS, Brasil (Freire, Kolling, Pereira)

Varieties	Recovery of strains - %							
	527		532		566		587	
	1st y	2nd y	1st y	2nd y	1st y	2nd y	1st y	2nd y
Planalto	25	-	30	-	-	3	45	95
Bragg	37	6	33	5	-	2	30	83
Bossier	15	17	12	6	-	3	73	62
IAS - 1	12	13	15	10	-	-	73	40
IAS - 5	38	9	12	7	-	9	50	68
S. Rosa	27	11	25	3	-	-	47	76
Prata	13	15	38	3	-	1	49	78
Davis	55	11	10	6	-	1	35	76
IAS - 4	34	1	45	6	-	3	21	81
Hardee	37	-	9	-	-	2	54	94
Perola	39	5	26	3	3	3	66	88
Pampeira	29	15	5	2	-	-	66	83
Means (%)	30	8	21	3	0,25	2	48	77

INOCULANTS AND INOCULATION

As has already been noted, work by Johnson, Means and Weber (1965) showed that the proportion of nodules produced by inoculation differed greatly according to rates of inoculum, methods of application and the established population of *R. japonicum*.

Burton (1975) has recently reviewed the literature on inoculants and listed six types available in the United States: 1. moist peat powder 2. liquid broth 3. lyophilized rhizobia in talc 4. oil-dried rhizobia in vermiculite 5. agar or bottle culture 6. granular-type peat for direct application to the soil. He emphasized the importance of using the most effective rhizobia available when soybeans are planted the first time, because ineffective bacteria can make a soil unprofitable for soybean production until a successful technique is developed to introduce a better strain and overcome the undesired population. We could add that ineffective rhizobia can be introduced in bad inoculants or can exist in the soil and have their population increased by no or inadequate inoculation with the first planting.

Table 4. Recovery of strains (%)* from a multi-inoculant by serological identification from 3 soybean varieties in relation to liming. J.Cast. RS (Kolling et al.)

Lime t/ha	Strains	Varieties					
		Planalto		Hardee		Bragg	
		1975	1976	1975	1976	1975	1976
0	532	1,6	1,1	0,5	1,1	6	7
	566	50	40	59	45	51	40
	587	39	52	30	49	33	49
	Neg.	9	6	10	1	10	3
3	532	1	2	1	0,5	5	10
	566	38	24	54	46	44	25
	587	55	69	40	48	44	62
	Neg.	5	4	4	4	6	1
6	532	--	4	0,5	2	9	7
	566	45	31	51	31	36	30
	587	43	59	39	62	10	58
	Neg.	11	5	9	2	2	3

* Strains recovery (%) from a multi-inoculant in 3 lime levels. J. Cast. RS (Kolling et al.)

In spite of the search for other types of substrates, moist peat inoculant has remained in greatest use because of its so far unmatched qualities. According to Vincent (1965, 1971) inoculant survival on seed was much better when applied in peat form rather than liquid, agar or bottle culture. Lyophilized inoculants have a good shelf life but survival on seed is poor. Besides the above mentioned papers, useful general information on inoculants can be found in Vincent (1958, 1970, 1971), Roughley (1970), and Date (1970). Freire (1964) and Freire and Jones (1963) investigated the effects of storage temperature, moisture and sterilization of the peat on the survival of *R. japonicum* in the inoculants.

One point deserving of more research is the rhizobia composition of inoculants. Multi-strain inoculants have been produced for a long time by industry as a means of covering a wider range of cultivars and environment conditions (Burton, 1967; Burton, 1975). Vincent (1965, 1970, 1971) has expressed opposition to multi-strain inoculants. Recently, Skrdleta (1973b, 1973c) and Caldwell and Vest (1970) have found

better performance of single-strain than multi-strain inoculants. However, one might present some questions: 1. when using a single-strain inoculant do we have relatively good assurance that it will colonize a soil under all possible environmental conditions in the producing areas and new areas of introduction and that it will not later on be dominated by less efficient strains? 2. will this strain have the same behavior with a wide range of commercial varieties? 3. would a mixture of highly efficient and competitive strains have a better chance to cope with adverse conditions, different varieties and different competitors? Vincent's observations that multi-strain inoculants have been insufficiently investigated is correct. However, the problem is to collect all the necessary information on the complex interelations of strains vs cultivars x environmental conditions. If, on the one hand the danger exists of a strain in a multi-inoculant being inefficient and more competitive on a variety on which the inoculum was not tested or which is cultivated in a region with environment conditions different from the nursery, this danger would also exist with a single-strain inoculant in relation to a naturalized population and to a different environment. Skrdleta (1973a) reported that competition between two mixed strains depended: 1. on the year of inoculation 2. directly on the ratio of cell numbers of the two strains and 3. on the moisture status as survival of one strain was statistically higher than the other in pots without watering.

Burton (1975) stated that normally 9 ml of slurry containing 4.4 g of peat inoculum is applied to 1 kg of seed and that survival and adhesiveness on seed can be improved by the use of 10-15% sucrose or maltose. Varma and Subba Rao (1973), however, recommended the use of only 5% sucrose because a higher percentage was harmful to the seed germination. There is, however, some controversy on the recommendation of the use of these products.

The use of water to wet the seed for more adherence of the peat powder or to make a slurry is a matter of complaint from many farmers because any excess of liquid will cause cracking of the seed coat, problems with the flow of seed in the planter and possibly with seed germination. Some farmers in Paraná, Brasil, have started to use kerosene instead of water and claim good results. Oliveira *et al.*, (1974) observed no harmful effect of kerosene on germination and Araujo and Oliveira (1974), though in preliminary research, found no reduction in nodulation when using kerosene and diesel oil for adherence of the inoculum on to the seed. These products would also have a repellent effect against the crown stem borer *Elasmopalpus lignoselus* which attacks seedlings and young plants.

Effect of Inoculum Rate

So many factors interfere with the success of the applied inoculum that the recommendation would be to apply the highest possible number of rhizobia per seed. However, from a practical standpoint it is important to have more precise information. Date (1970) states that

for most inoculated legumes experience has shown that 100 rhizobia per seed is sufficient for nodulation under favorable conditions. However, 100 times that figure would be needed if adverse conditions would exist for survival multiplication or competition with the indigenous population. In Australia standards for newly manufactured inoculant request a minimum of 3,000 rhizobia per seed and the inoculants have usually many times this requested minimum; for soybean the minimum standard for inoculant at sale is 2×10^6 rhizobia per gram of peat. In Brasil a federal law is requesting a minimum of 10 million cells per gram at sale which would give an average of 7,000 cells per soybean seed. Therefore the manufacturers must provide an inoculant 5 to 10 times that figure at the factory. Due to problems in transportation and storage a high percentage of the samples taken at planting time is below the requested standard. To be on the safe side the recommendation to farmers in the state of Rio Grande do Sul (50% of the Brasilian soybean planting in 1975) is to use double doses, that is, 400 grams of inoculant per 60 kg of seed when soybean is planted for the first time in the soil. Where the soil is hot and dry, which is a common occurrence in late plantings, or where the soil has not been limed, the recommendation is also to increase the quantity of the inoculum.

Burton (1975) recommends that a high quality inoculant must furnish 10^5 to 10^6 viable cells per seed. At least 2×10^5 rhizobia/seed are needed for effective, efficient nodulation under moderately good conditions and more inoculum is needed under less favorable conditions. In an earlier field study Burton and Curley, (1965) compared inoculum rates from 50,000 rhizobia/seed (standard rate) to 500,000 rhizobia/seed, in liquid and peat-base form. A slurry was added containing 6% sucrose to reduce the harmful effect of drying. The seeds were kept 1 to 21 days before planting. The conclusions were: 1. peat-base inoculant is superior to broth at all time periods; 2. broth is of low effectiveness after 7 days of storage; 3. pre-inoculation of seed no more than 3 weeks in advance appears practical providing a good peat-base inoculant is used and the seed is kept under cool non-drying conditions until planting; 4. effective nodulation under field conditions depends not only on a large number of live rhizobia, but also on conditions favorable to growth of the rhizobia cells on the seed; 5. emphasis must be placed on assuring maximum benefits rather than on ease of application.

The large volume of research attempting to overcome the naturalized *R. japonicum* population by massive inoculation has met with relative success. Weaver and Frederick (1974) used liquid inoculum levels up to 1 billion cells/2.5 cm of row in a greenhouse study with 22 soils containing *R. japonicum* from 2 to 1 million/g of soil. Numbers of nodules were not increased when the soil contained more than 1,000 rhizobia per gram of soil. At this or higher population levels plants are not likely to be extensively nodulated by inoculum applied at normal rates (less than 10,000 cells/seed). In a field study (Weaver, 1974), with six soils and inoculum up to 3.3×10^8 cells/2.5 cm of row and soil populations ranging from 1.2×10^1 to 2.3×10^5 rhizobia

per gram of soil, numbers of nodules increased in soils with 12 rhizobia/g or fewer when inoculum was applied at the level of 3.3 x 10^4 cells/seed. No increase was observed in soils with more than 1 x 10^3 rhizobia per gram. From the results there was an indication that: 1. to produce 50% or more of the nodules an inoculum rate 1,000 times the soil population must be used: 2. commercial inoculants in the US are probably not furnishing adequate numbers of rhizobia for successful nodulation competition on land previously cropped to soybeans. Kapusta and Rouwenhorst (1973) were successful in achieving population shifts only at ultra-high inoculum rates. They indicated that if superior strains are developed in the future the technique of massive inoculation would be recommended to have them established rapidly. We could suggest the following procedures to be followed before trying field experiments for introducing strains into a contaminated soil: 1. screening for efficient strains comparing the predominant strains in a region against highly efficient strains from other sources; 2. screening for ability to compete for nodule sites between predominant strains and the more efficient strains using different cultivars in greenhouse pots with the types of the soils of the region, trying to find more competitive long-term efficient strains with the varieties under use and also to find cultivars on which the predominant strains would have less competitive ability; 3. long-term (for several years) field experiments with the strains selected, changing soil environment (as by liming), inoculum rates, methods of application and cultivars.

Burton (1975) indicated that the early nodulation of the primary root signifies inoculation success. A seedling may have 5-7 effective nodules on the primary root two weeks after planting. Poorer inoculation with ineffective strains or low numbers of cells in the inoculum will lead to fewer tap root nodules and many nodules on lateral roots at 3-4 weeks. Nodules on the primary root at two weeks is a good criterion of inoculation sufficiency. High temperature of storage of the inoculated seed before planting is harmful for the survival of the bacteria. Seedlings had more tap root nodules when the seeds were stored at 22°C than at 32°C. Burton also found that numbers of nodules declined with time of storage and were higher with peat inoculant than with inoculum in liquid form. In hot, dry soils peat inoculum gave better results than the liquid form. In another experiment in Leonard jars at greenhouse, inoculum ranged from 0 to 375 x 10^3 rhizobia/seed and there were no differences at 15 x 10^3 and higher rates. Nitrogen percentage and total nitrogen were directly related to the number of rhizobia per seed. Critical time for evaluation of inoculum sufficiency appears to be when plants are 22-24 days old and the importance of differentiating between inoculum sufficiency and rhizobia effectiveness is noted. Inoculum sufficiency is concerned with adequate numbers of rhizobia to bring about effective nodulation. On the other hand, rhizobia effectiveness can be best determined by growing plants to maturity to create maximum nitrogen stress.

Effect of Adverse Conditions on Inoculation

Many soil factors will interact to influence rhizobia survival, root colonization and competition for nodules sites. There is evidence that, when the inoculated seed is planted in soil, adverse conditions may be extremely harmful to survival and development of the bacteria and thus to the sufficiency of nodulation. When the soil is being cropped for the first time or the *R. japonicum* population is low, the loss in nodulation and in the seed yield may be high. In tropical and sub-tropical regions, soil temperature and soil moisture are important factors affecting the fate of the rhizobia on the inoculated seed. Results of research on these factors are found in several papers (Masefield, 1958; Kuo and Boersma, 1971). Plantings are made in late spring and early summer when high soil temperatures and lack of rainfall are common. These conditions sometimes delay germination for several days, even 1-2 weeks. Nodule number and efficiency are then affected depending on the intensity of these stress conditions and on other adverse factors such as deficiency or phosphorus, high acidity and toxic levels of aluminum and manganese.

In many areas maximum surface soil temperature in summer greatly exceed the optimum temperature for symbiotic N_2 fixation. In a greenhouse experiment, Wilson (1975) observed that at moderate temperature neither inoculation depth nor surface soil moisture affected the amount of nitrogen fixed. However, at relatively high temperature, plants in low soil moisture treatments fixed less than 25% of those with high moisture. A decrease in fixation was also observed with deeper inoculation at high temperature. In a greenhouse experiment Galleti, *et al.*, (1972) found that diurnal maximum soil temperatures above 33°C are harmful to nodule initiation and efficiency. After initiation, effects on development and N_2 fixation are less pronounced. Strains of *R. japonicum* and varieties differ in tolerance to soil temperatures. Scudder (1975) refers to the harmful effects of high soil temperature on survival and nodulation of *R. japonicum* in tropical and sub-tropical regions. Inoculum is subjected to high temperature, sunlight and drying before planting, high temperature in the planter hopper during planting, and hot, dry soil after planting. In Florida a soil temperature of 42°C at 4-cm depth is common. In field experiments performed in a year when the conditions were not very hard as there was good moisture in the soil, inoculum placed in the seed furrow or at depths of 2.5 to 5.0 cm below the seed gave significant differences in root nodulation, plant color and soybean yield. Inoculum placement in granular or liquid form gave equal results but, when inoculum was applied by the regular seed coating or stirring it into the hopper box, results were not safisfactory.

Stress conditions acting upon the bacteria and/or the plant might limit the performance of the inoculum, inhibit further nodule formation and/or adequate N_2 fixation by the nodules that have been formed. Thus, the adverse factors may affect nodule formation or nodule functioning or

both. Sometimes, when the restriction is only on early nodule formation a compensating mechanism operates in which the nodules grow beyond the usual size and the plant is supplied with enough nitrogen. Anticipation of one or more of the stress conditions and adoption of adequate counter measures can sometimes be of value in minimizing the harmful effects. In hot, dry soils for instance the use of massive doses of inoculum and/or deep placement of the regular inoculum or use of granular inoculant will assure better nodulation. And although the attainment of high productivity requires adequate correction of pH and toxic factors the use of massive doses of inoculant might also enhance nodulation, nitrogen fixation and yield. The problem of overcoming a high indigenous population remains however unsolved as there is no practical way yet to introduce and maintain a more efficient strain in the soil.

Freire *et al.*, (1974) investigated the influence of levels of inoculum vs 3 levels of lime in a field experiment (soil pH 4.8, Al 1.2 me/100g, Mn 31 kg/ha). Naturalized *R. japonicum* was low. Without lime, only the highest level (400 x 10^3 cells/seed) gave marked yield increase, but with lime the first inoculum level (4 x 10^3 cells/seed) was sufficient for nodulation and maximum yield. Liming stimulated the naturalized population, but the first inoculum level increased further the number and weight of nodules with a small increase in yield in relation to the control (limed, no inoculation). In another field experiment (Freire *et al.*, 1975), the soil had a low *R. japonicum* population and was limed to pH 5.6. The seeds were inoculated with up to 20 x 30^3 cells/seed. Without lime there was a small, non-significant increase in nodules weight and seed yield due to the first inoculum level (5 x 10^3 cells/seed). With lime, however, there were no differences between any of the treatments due to the stimulation of the naturalized population. Reskasem (personal communication) found that the effect of soil acidity on nodulation is highly dependent on the number of rhizobia cells in the inoculum.

CONTRIBUTION FROM SYMBIOTIC DINITROGEN FIXATION

Most of the soils where soybean cultivation has expanded from its origin in Asia were originally devoid of its quite specific *Rhizobium japonicum*, the contrary occurring with bacteria from the cowpea type which is very common mainly in the tropical and sub-tropical regions. Leonard, according to Vest *et al.*, (1973), had early established that some strains of *R. japonicum* would induce nodulation on other legumes, mainly on cowpea, but on the other hand only a few of the *Rhizobium* from cowpea are liable to form nodules on selected soybean varieties. Thus, the practice of inoculation for the establishment of *R. japonicum* populations has played an important role in the expansion of soybeans to new areas of the world, and has contributed on a large scale to decreased fertilizer nitrogen requirements and to increased yields.

Vest *et al.*, (1973) state that inoculation of crop land with

R. japcnicum in the north-central US was not completed until after World War II and that in 1944 only 77% of the land in Iowa had plants with good nodulation. After several crops, further benefits from inoculation are less likely. However, a survey in a soybean region pointed out that 15% of the sites sampled had insufficient populations of *R. japonicum* for adequate nodulation and this occurred even on land planted with inoculated seed a month earlier. Significant difference in yield, oil, percentage of protein, weight of nodules per plant, leaf color and fresh plant weight were associated with inoculation with different strains in soils devoid of *R. japonicum*, but little or no response is usually obtained in soils with the bacteria. According to Caldwell and Vest, as cited, the environment influences both strain and variety performance each year, but in general a strain associated with high yields on most varieties one year is likely to give a similar performance in other years. Caldwell claims that difference in green weight of the plants is not always a good measure of seed yield and suggests the measurement of the differences in N_2 fixing ability of the strains under test at different periods of the growing season.

Johnson and Hume (1973) have found high correlation between each pair of the following parameters: nodule leghaemoglobin, acetylene reduction and nodule weight. Döbereiner *et al.*, (1966) claimed to have found significant correlation between total nitrogen in plant and nodule dry weight which agrees with results of other workers. In Döbereiner's work, the regression coefficient was not affected by liming, fertility, pH, soil temperature or plant variety; one completely inefficient strain and fertilizer nitrogen were the only factors which affected the regression coefficient. In another study Döbereiner *et al.*, (1970) found similar correlations with 24 strains, and three of these presented twice the average efficiency per unit of nodular tissue and they were called "exceptionals".

Soybeans have a high nitrogen requirement and from the three sources of N available to soybeans, soil, fertilizer and atmospheric N_2, each one could possibly supply the needs of the plants. By the use of new techniques available today such as 15 N, acetylene reduction and non-nodulating vs nodulating isolines, the measurement of the contribution from symbiosis is much more reliable than before. DeMooy *et al.*, (1973) reviewed several of the more recent papers on the subject and cite contributions ranging from 13% of the total N in dry seasons to 74% with the soil N imobilized by ground corn cobs, with an average of 25-30% under normal conditions. A 40% contribution in a favorable season corresponded to 78 kg N/ha for a 2,690 kg/ha seed yield. A total fixation of 94 kg N/ha was determined by Kjeldahl and ^{15}N techniques and 74 to 81 kg N/ha by C_2H_4 assay.

The contribution of fixed N to growth and yield of soybeans depends on many factors: 1. availability of soil or fertilizer nitrogen; 2. existence of ineffective and/or inefficient native *R. japonicum* population; 3. soil moisture; 4. other nutritional and environment factors. Vest *et al.*, (1973) mentioned the work of Weber using nodulating genotypes; the nodulated one produced 2,800 kg of seed per hectare

and fixed 84 kg N/ha corresponding to 40% of the total N. The quantity fixed and the ratio of fixed N to total N nearly doubled when soil N was imobilized with corn cobs, but the yield remained the same. They also mentioned the work of Vest in which subtraction of the total N in uninoculated nodulating plots from total N in mixtures of non-nodulating and nodulating genotypes indicated an average of 121 kg N/ha fixed, which was 52% of total in beans of the variety Delmar; and of Hardy *et al.*, who found a fixation of 148 to 163 kg N/ha by integration of the acetylene reduction for a growing season for the variety Kent; and of Sloger, with 164 kg N/ha for the same variety. When the new techniques were not available earlier estimates of N_2 fixation for soybeans were much lower: 53-64 kg N/ha.

According to de Mooy *et al.*, (1973) comparisons using non-nodulating and nodulating isolines are not quite valid due to the differences in the root systems. Non-nodulating isolines would have a more developed root system which would correspond to a significant effect on absorption of phosphorus and other nutrients. However, this criticism could also be applied to the work comparing inoculated and non-inoculated plants; under greenhouse or field conditions non-inoculated plants usually have a much more developed root system.

In a field experiment Harper (1975) found a N fixation contribution of 80 to 100 kg N/ha and even 160 kg N/ha using the N_2 (C_2H_2) assay. He mentions a seven-year study of Havelka who found an average of 25% from fixation and contributions ranging from 10 to 59% from other reports. Johnson *et al.*, also mentioned by Harper, stated that an average of 25 to 50% at the total nitrogen should be expected from N_2 fixation under normal field conditions with non-nitrogen deficient soils and that this would indicate that soil nitrogen is the primary source of nitrogen and N_2 would be the second.

Sloger (1969) used acetylene reduction assay and found evidence that the interaction of soybean vs *R. japonicum* in some way controls nitrogenase activity which results in a particular growth response by the plant. Klucas (1974) found that on one variety specific nitrogenase activity decreased abruptly between 18 to 65 days after planting and on the second variety 68 to 75 days after planting. However, major changes in dry weight, total nitrogen and leghaemoglobin levels during the period were not detected. In a study of the development of nodulation and N_2 fixation on several soybean varieties, Ruschel and Reuszer (1973) found that maximum weight of nodules occurred 80-95 days after planting N_2 (C_2H_2) fixation was highest 65 days after planting for the early varieties and 15 days later for the late varieties. There was indication that fixation followed the development of the plants and decreased with maturity. Nodule weight per plant was negatively correlated with the production of ethylene for young plants and positively correlated for the old plants. This would indicate that N_2 fixation is a function of plant development.

In practical terms, responses to inoculation under field conditions have been from nil to very high. In field experiments in the State of Rio Grande do Sul, Brasil, Freire *et al.*, (1969) compared eight

varieties inoculated with a multi-strain inoculant. Yield responses ranged from 6 percent to 152 percent over a non-inoculated control. In an experiment with four strain vs four varieties, the effect of strain varied from 0 to 44 percent over non-inoculated plots, the best strain increasing yield from 1467 kg/ha to 2115 kg/ha. In another trial, with four inoculants vs six varieties, the best inoculant gave a 4 percent yield increase over non-inoculation on variety Hill (2272/2363 kg/ha), 65 percent on variety Bienville (1788/2950 kg/ha) and 67 percent on variety L-2006 (1383/2322 kg/ha). Inoculating of seeds in limed soil increased yield 30 percent over non-inoculated whereas in unlimed soil the increase was only 8 percent. The existence of an indigenous population of *R. japonicum* limits the response to inoculation. In the State of São Paulo, five varieties were inoculated with different *R. japonicum* strains. In spite of the fact that the soil had never been cropped to soybeans, there was no response to inoculation due to generalized nodulation (Mascarenhas, 1968).

EFFORTS TO INCREASE THE LEVEL OF THE YIELD PLATEAU

Research has resulted in marked increases in the yields of several important crops, such as corn, rice and wheat, mainly through breeding and high response to nutrients. However, with soybeans the success has been limited in spite of the extensive work done. Photosynthesis through the less efficient C_3 pathway and the loss of a large part of the fixed CO_2 (10-50%) through photorespiration account for the reduced supply of carbohydrates and therefore, for the limitation in N_2 fixation and also for the little responses to extra mineral nitrogen in the soil. If one considers the contributions from N_2 fixation mentioned before and the highest fixation rates obtained under abnormal experimental conditions, the evidence is convincing for the existence of one or more relatively unknown and uncontrolled factors which limits N_2 fixation under field conditions.

The tentative indications of increased soybean yield by the supply of nitrogen fertilizer applications are numerous, and a great deal of research is being done along this line. Some authors have met with some success and have expressed the opinion that soybeans can use more nitrogen than is provided by the soil and symbiosis (de Mooy *et al.*, 1973). Bezdicek *et al.*, (1974) applied nitrogen on a previous rye cover crop and found that NH_4NO_3 was better than urea and gave significant increases in dry matter, N uptake and yield. Miller *et al.*, (1973), studying 16 strains on 3 varieties at varying nitrogen levels found highly significant yield increases for two years and a highly significant decrease in one year. The highest yield occurred from inoculated plots at all N levels and the interaction of soybean variety vs nitrogen rate was highly significant for the three years. Tanner, Hume and Criswell (personal communication) indicated that yield response could be achieved through placement of anhydrous ammonia below the nodule zone and also that promotion of nodulation and increase in yields was obtained by the incorporation of 9 tons of organic matter into the nodule zone.

They conducted two field experiments to determine the effect of supplemental nitrogen at time of declining nodule activity (end of flowering) on nodulation, seed yield and protein of two varieties. The extra nitrogen reduced acetylene reduction activity for both varieties, increased seed yield and seed protein content for one variety and seed protein content and vegetative protein yield for the other variety. Ham *et al.*, (1975), in a two-year study used one commercial variety, a nodulating - non-nodulating pair of isolines, fertilizer nitrogen up to 224 kg/ha and fertilizer sulphur. Forms of fertilizer nitrogen were ammonium nitrate, urea, urea plus S and two slow-release fertilizers: S-coated urea and urea-formaldehyde. Nitrogen fertilizer increased seed yield, weight of seed, and percentage of seed protein. All sources of N reduced N_2 fixation, nodule weight and number, and weight per nodule. They concluded that N_2 fixation failed to supply amounts of nitrogen essential for maximum seed yield and percentage of protein and presented the following possible explanations for positive reactions: 1. The plants with extra nitrogen received a better start, since N_2 fixation occurred at the 14th day after planting; 2. if adverse environmental conditions delay fixation even more the influence from added nitrogen may be higher; 3. poor nodulation or nodulation by lower efficiency combinations of strain x variety may also account for the response to nitrogen fertilizer; 4. nitrate fertilizer was more detrimental to nodulation than ammonium. Hatfield *et al.*, (1974), in an experiment with a gravel culture system, found evidence of the importance of soil nitrogen for the initial growth of soybeans even with adequate inoculation. Harper (1975) indicated that, by comparison of seasonal nitrate assimilation (nitrate reductase activity) with seasonal symbiotic N_2 (C_2H_2) fixation, nitrate uptake and metabolism are of primary importance during vegetative growth and flowering while N_2 fixation becomes more important during pod filling.

De Mooy *et al.*, (1973) reviewed the extensive literature up to 1971 and reached the conclusion that the causal mechanism for the reduction in the symbiotic process in the presence of combined nitrogen is unknown. This inverse relationship between mineral nitrogen and symbiotic nitrogen could be explained by the fact that photosynthates are in limiting amounts and may supplemental nitrogen absorbed from soil or fertility sources combines with photosynthates that otherwise would be available to react with the nodule-fixed N_2. Field trials have demonstrated this inverse relationship is dependent on seasonal conditions. One study in a poor season showed that the symbiotic fixation contribution was reduced from 16% to 2% by the application of 168 kg N/ha; under good growth conditions the contribution was reduced from 40% to 16% and from 72% to 6% when soil nitrogen was partially imobilized. The effects on nodulation are striking. Nodule number and size are usually reduced when soil nitrogen is increased. Some inconsistent effects on nodulation are due to differences in the substrates used (nutrient solutions or soil cultures), varying initial nitrogen levels and actual availability of nitrogen. Under field conditions rainfall and temperature are the main factors affecting nodulation. In a hot, dry season 112-224 kg N/ha caused a reduction of 30-90% in

nodulation, but with adequate rainfall and temperature the reduction was only 35%. The prevalent environmental conditions are responsible for the extremely variable results. The nitrogen requirement of the plants under field conditions are more or less satisfied by soil nitrogen and fixation. However, stress moisture conditions would limit the mineralization of organic nitrogen and nitrification and so a positive response to nitrogen fertilizer would be liable to occur mainly at two critical peaks, at rapid vegetative growth and during pod filling. Lyons and Early (1952) in a two year field experiment found marked yield responses to added nitrogen in the hot, dry season but not with adequate rainfall and cooler temperature, which they explained as having a direct influence on the sufficiency of symbiotic fixation for maximum yields. It is our own experience that even in soils of low organic matter high peaks in nitrate occur with adequate moisture and the initiation of nodules and fixation is then delayed by one or two weeks. On the other hand, heavy rainfall will leach nitrates and less interference with nodulation would be expected.

Critical periods for nitrogen application according to some authors would be just prior to flowering when the soybeans needs more than fixation and soil can supply. Better growth, larger leaf area, more abundant flowering and higher yield, have been reported for nitrogen applications at pre-bloom stage. According to others, reduced food supply from the host leads to less symbiotic activity during pod filling when nitrogen is still required. However, a controversy seems to exist concerning the extension of the fixation process which would be influenced by varied experimental conditions. This subject will be treated further at another point of this paper.

The research on alternative sources of combined nitrogen shows that NO_3^- is the most detrimental to nodulation. In relation to NH_4NO_3, HNO_3 and NH_4OH, urea seems to be better (de Mooy *et al.*, 1973). Nodule initiation, development and function are all depressed by nitrates. Ruschel *et al.*, (1974), comparing several sources of mineral nitrogen (sodium nitrate, ammonium sulphate, ammonium nitrate and urea) found that nodulation was highest in the treatment with urea. Harper (1975) mentioned that recent evidence has indicated that urea has little if any inhibitory effect on nodule formation and function of soybeans in hydroponic culture provided that there is no nitrification to nitrate. He also mentioned the work of Sprent which would indicate that urea is taken up more slowly than nitrate and this would explain the existence of better nodulation. Harper also pointed out that the use of urea as a nitrogen source is quite attractive from the standpoint that uptake of urea and dissociation of NH_4 and CO_2 would provide a reduced source of N with much less energy expendure by the plant compared with reduction of either NO_3 or N_2. Johnson, Kurtz and Welch (personal communication) in a study with $Ca(^{15}NO_3)_2$ found that: 1. as fertilizer nitrogen increased from zero to 448 kg N/ha, nitrogen from fixation was reduced from 48% to 10% of the total nitrogen; 2. the fertilizer contribution increased from 0 to 57% and, 3. soybean can remove as much nitrogen as corn at low soil N level and more at high levels.

In a study by Weber, referred to by de Mooy *et al.*, (1973) nodulating isolines always had higher yield which could be explained if the symbiotic nitrogen were more desirable or if the energy required for symbiosis and reduction of the fixed nitrogen were less than that required for nitrate absorption and reduction by the non-nodulating isoline. In recent years, that review points out responses of 130 to 670 kg/ha from the application of 112 to 224 kg N/ha respectively have been obtained. In 21 experiments seven gave positive response in three years. According to those authors, positive responses would be caused by: 1. the strains of *R. japonicum* populations in the soil, as it is possible that some strains can fix N_2 at a higher nitrogen applied level than others; 2. if molybdenum is in short supply symbiotic fixation is limited. To these explanations we could add the ones mentioned before from the review of Ham *et al.*, (1975) and also the ones related to the seasonal conditions as temperature and rainfall. In an experiment with non-nodulating vs nodulating isolines under humid, sub-tropical conditions, Pal and Saxena (1975) concluded that the non-nodulating isoline needed 235-300 kg N/ha to give a yield equal to the nodulating isoline and that symbiosis proved better for nitrogen nutrition of soybeans.

Deep placement of nitrogen fertilizer was not as detrimental to nodule development as was uniform distribution of the fertilizer (Harper and Cooper, 1971). There was a reduction in nodule fresh weight and haemoglobin by nitrogen fertilization but not in nodule number. Nodulation response was not strictly dependent upon internal nitrate content of the roots. They concluded that control of the application site may provide a means of alleviating the detrimental effect of combined nitrogen on nodulation.

Beard and Hoover (1971) applied burned or shredded straw, and 0-168 kg N/ha as ammonium sulphate at planting, at flowering, or at both times. Even though plants at the no-nitrogen rates were yellow green in the early growth stages there were no significant differences in yield from any of the treatments; nodule number was linearly and inversely related to rate of nitrogen and the reduction was more marked at planting time applications than at flowering. With the shredded straw, nodulation was enhanced. Welch (1974) found no response to manure up to 80t/ha applied for seven years to corn and planted with soybean in the 8th year. Also there was no response to direct nitrogen application (0-40-120 lb/A-year) at different planting dates and N rates, neither to side-dressed application (0-100-200 lb/A at flowering and at pod-filling stages, nor to plow-down or disked in applications. In another experiment with nitrogen rates as high as 1600 lb/A, there was one case of response to 400 lb N/A. The same author found significant yield increases in only three out of 133 instances using different rates, times and method of nitrogen application, direct or residual, organic or inorganic and in several locations. The three positive examples occurred at high uneconomical rates of fertilizer and he concluded that nitrogen availability is not the growth factor that is presently limiting soybean yield in Illinois.

These results are in accordance with the review paper of

de Mooy *et al.*, (1973) in which they conclude that good response to applied N occur in years of low average yield and are larger under irrigation, but the most frequent report on nitrogen fertilizer application in the United States is a lack of response or a non-profitable increase in grain yield. In some states of US, nitrogen fertilizer is recommended to accelerate early growth and facilitate cultivation. In URSS a yield response of 500 kg/ha has been recorded and nitrogen fertilizer is recommended more often, with better results from applications at bloom and seed formation. Also the responses have been associated with higher rainfall and podzolic soils of low fertility but sometimes in soils high in phosphorus and calcium. In regions of low rainfall in that country nitrogen is considered more important than phosphorus and potassium, but the response is small, between 80 to 120 kg/ha and usually in areas of weak nodulation caused by low populations of *R. japonicum*. In Brasil, most of the soybean cropped soils have originally low to extremely low pH which in some cases is associated with toxic levels of aluminum and manganese. Inadequate liming and fertilization in these soils brings very poor nodulation (Freire & Vidor, 1970) and nitrogen application is likely to give highly significant responses.

Grimm and Fole (unpublished information) found a marked yield increase of 652 kg/ha from the application of 120 kg N/ha without lime; however at the lime levels of 4.4 t/ha and 8.8 t/ha, half and equal to the quantity of lime recommended to reach pH 6.5, the responses were small or absent., Number and weight of nodules, measured however only at zero and at the highest level of lime, were markedly increased by liming which also benefited the native population. Unfortunately, most of the research on nitrogen application lacks such secondary but important observations as nitrate availability in the soil and sometimes even a nodulation evaluation.

From a seven-year experiment with non-nodulating vs nodulating isolines, Weber (1966) concluded that yields were essentially the same when enough combined nitrogen was available. The amount of N_2 fixation depended on available soil nitrogen, water or both and ranged from 1 to 142 lb/acre, corresponding to 1 to 74% of the total nitrogen uptake. Nodule weight, number and size were directly related to increased N_2 fixation and inversely related to increased increments of applied nitrogen. With no nitrogen application the residual effect of a nodulated soybean crop in the previous year corresponded to 33 pounds of nitrogen per acre.

Chesney (1973), in Guyana and Herrara and Longeri (1974) in Chile found no response to nitrogen application. In Brazil, Rios and Santos (1973) had a grain yield response of 325 kg/ha over the control from an application of 120 kg N/ha. Number and weight of nodules was not affected at 40 and 60 kg N/ha levels but was decreased when 120 kg of nitrogen was applied. Miyasaka (1970) found no effect from nitrogen application in a soil with native *R. japonicum*, but inoculation still gave a yield response of 250 kg/ha without lime and 332 kg/ha with lime. Dobereiner *et al.*, (1966) in a greenhouse experiment found that nodulation

and N_2 fixation in one variety was affected at a higher level of N applied than in the other variety and they concluded that this different behavior in relation to nitrogen fertilizer was due to physiological differences in the metabolism. Barni *et al.*, (1975) used ammonium sulphate up to 128 kg N/ha at planting time and found that there were no differences from nitrogen applications and that there was an inverse relation between nodulation and fertilizer levels.

Harper (1974) established field and hydroponic studies to determine relative potential of soybeans to utilize nitrate and atmospheric nitrogen. Maximal nitrate utilization occurred at the full bloom stage. N_2 fixation measured by acetylene reduction had its highest peak some three weeks later during pod-filling. Seed yield of plants totally dependent on N_2 was less than one half of the yield of those utilizing both nitrate and atmospheric N_2 hydroponic conditions. Plants at low NO_3 had higher symbiotic fixation than those with no nitrate. Similarly seed yield of plants on high NO_3 which inhibited fixation was lower than of plants utilizing both NO_3 and N_2. Thus, symbiotic N_2 fixation and nitrate utilization both appeared essential for maximum yield.

Hardy and Havelka (personal communication) concluded from their work and from that of others that research aimed at overcoming the nitrogen barrier or non-productive trade-off (between soil and fertilizer nitrogen and N_2 fixation) has not yet found a major break-through and suggest the following approaches: 1. utilize forms of nitrogen fertilizer that do not inhibit N_2 fixation; 2. develop soybean cultivars that would respond more to nitrogen fertilizer; 3. develop *R. japonicum* strains insensitive to nitrogen fertilizer. To the above suggestions we could add: 1. soybeans planted very late have a shorter period to grow, and then nitrogen could be expected to increase yield, or at least plant height which would facilitate harvest and reduce losses; 2. more information on the interaction of lime levels vs N on several soil types and under different environmental conditions would also be valuable in areas of low lime availability.

The efforts to increase the response to supplemental nitrogen have declined after the rise in nitrogen fertilizer prices in consequence of the oil crisis. Now most efforts of research are on increasing the contribution from N_2 fixation and on the physiology of the soybean plant in relation mainly to carbohydrate availability. In a study by Weil and Ohlrogge (1975) there were plants at normal density and plants thinned to one plant/m^2. Number and size of nodules increased to early pod filling and then declined. The thinned plants showed significantly more nodule volume/plant, greater percentage of red nodules and less green nodules during pod filling than plants at normal density. The thinning treatment apparently postponed the decline in nodulation for several weeks and this was attributed to the availability of more photosynthates.

Holl and La Rue (1975) comment that in spite of the early recognition of the contribution of the host genotype to N_2 fixation, legume breeders have not exploited the potential. There is still ignorance as

to the genetic features of the infection and the fixation processes. Breeders have seldom consciously selected for desirable dinitrogen fixing properties and the N_2 fixing capacity of new lines is not evaluated. It is of importance to consider that the host is involved in the process of infection, nodule development and fixation. In soybeans four genes affecting symbiosis have been described but only one has been studied. So mutations could alter the symbiotic association and there appears to be no reason why symbiotic fixation cannot be increased by genetic means. The following approaches are suggested: 1. select cultivars that nodulate early under harsh soil conditions; 2. select cultivars that would fix N_2 in the presence of high soil nitrate levels and continue to fix throughout their life; 3. breed for more effective photosynthesis, decreased photorespiration, delayed lodging and modified pod-nodule competition for carbon. Ogren and Rinne (1973) recommended the screening of the progeny of mutagen-treated soybeans for photorespiratory deficient mutants. Trying to reach the other partner, microbiologists have attempted to find more effective bacterial strains but little success has been attained in introducing novel strains into areas already populated by *R. japonicum*. Johnson *et al.*, (personal communication), working with the pea symbiosis, found considerable variation in nodule structure, infectiveness and effectiveness when different isolates are used to inoculate different varieties of peas. They suggested two approaches in the isolation of bacterial mutants of increased N_2 fixation: 1. isolation of rhizobia mutants altered in simple respects and testing for symbiotic capacity; 2. testing of unselected survivors of a mutagenic treatment.

Lawn and Brun (1974) tried to modify the supply of photosynthates by supplementing light, shading, depodding and defoliating. They reached the following conclusions: 1. total acetylene reduction activity per plant increases during flowering, reaches a maximum near the end of flowering and then declines markedly during early pod filling; 2. treatments to enhance the photosynthetic source-sink ratio (supplemental light and depodding) maintained nodular activity well above the control, and shading and defoliation decreased nodular activity, 3. symbiotic fixation in the varieties tested declined during pod filling as a result of inadequate supply of photosynthates to the nodules. Rocha *et al.*, (1968) also worked on the effect of light on N_2 fixation. Dry weight of nodules, dry matter of plants and fixation were positively affected by increase in the intensity of solar radiation. Total nitrogen in the plants varied linearly with the dry weight of nodules independently of shade. Hardy and Havelka (1974, 1975) obtained a N_2-fixation increase from 112 kg/ha to 448 kg/ha by increasing the CO_2 availability to the plants proving therefore the important role of the photosynthates as a limiting factor for fixation. The increased supply of CO_2 could also explain the increased fixation obtained when a high carbon material, such as straw or corn cobs, is incorporated into the soil with the objective of imobilizing soil nitrogen (Freire, 1965). It is now known that: 1. nitrogenase concentration need not be the limiting factor for N_2 fixation; 2. plant variety is more important than bacterial strain in determining the fixation characteristics

(age of initiation and loss of exponential phase), rate of increase and total N_2 fixed; 3. N_2 fixation may provide only about 25% of the total N in the mature plant; 4. photosynthate is a major limiting factor for N_2 fixation; 5. a photosynthate-rich plant prefers N_2 to soil N and increase of photosynthate is the first experimentally demonstrated key to overcoming the N-input barriers. Hardy and Havelka (1974, 1975) examined the factors that affect photosynthate availability to the nodule and documented its role as a major limiting factor for N_2 fixation.

Streeter (1972) measured the quantity of nitrogen compounds in soil and in the stem exudates of plants under field conditions. Nitrate content in the soil and stem exudates was highest during growing stages preceding flowering. After flowering, amino acid concentrations in the exudate were many times the nitrate concentrations and this relationship was not affected by the fluctuations in soil nitrate. Asparagine was the predominant amino acid. Streeter (1973, 1974) claims that experimental results show that: 1. nodule bacteria can supply 30-60% and even 100% of the nitrogen needed by the soybean crop; 2. there is no practical way to increase the overall utilization of nitrogen by application of nitrogen fertilizer; 3. N_2 fixation is closed linked to other nutritional systems in the plant; 4. something supplied to the roots by the tops of the plants would possibly limit the rate of fixation. He succeeded in doubling the shoot: root ratio by grafting and found a great increase in the N_2 fixation, and this increase was higher and sustained for a longer period when the grafting was accomplished during the seed formation stage. After some time nodule weight increased and acetylene reduction declined to near the control rates. He concluded that nodulated soybeans are capable of fixing nitrogen at rates greater than those which normally prevail.

The great majority of the work done so far to improve N_2 fixation in soybeans has been through work on the bacteria and little or no success has been obtained except for the selection of more efficient already existing bacteria genotypes. However, as already indicated by some workers, the plant is the easiest partner to work with to attain success. Another point is that besides the approach followed by some workers who have been trying to introduce more competitive and more efficient strains into the old soybean areas, we think that the feasibility of development of cultivars more adapted to the strains of the naturalized populations should also be examined.

Devine (personal communication) claims that the challenge of increasing symbiotic fixation in soybeans may be considered from two aspects: 1. development of symbiont combinations more productive than those in use; 2. development of methods of successfully establishing improved rhizobia strains against competition from indigenous strains. Genetic control of infectivity specificity could be a solution for this establishment. Ninety percent of the *R. japonicum* field population does not infect soybean plant having the genetic factor rj_1 in homozygous recessive condition. However, some strains have exhibited restricted infectivity with rj_1 genotype in soil in pots. If this infectivity could be enhanced and combined with improved capability for fixation it should be feasible to establish an improved host cultivar vs inoculated

rhizobia strain relationship in the field environment by genetic means. Wacek and Brill (personal communication) have developed a simple quantitative assay for screening for N_2 fixing ability in soybeans which seems to be very useful for this purpose. This test is based on the growth of the soybean plants in small vials in the greenhouse, cutting off the plant tops, stoppering the vials and running the acetylene reduction assay by direct introduction of acetylene into the vials without disturbing the roots.

SOME FACTORS LIMITING N_2 FIXATION

Many areas in tropical and sub-tropical regions have highly acid soils sometimes with toxic levels of exchangeable aluminum and manganese. In some areas the loss of the forest cover and an extractive approach to agriculture explain the present situation. In some other areas the soil evolution, interelated with climate, did not allow the formation of a dense vegetal cover, and the intense weathering has depleted the soil of almost all nutrients and resulted in increased acidity and toxicity. This is the case of the "cerrados" areas in Brasil which are similar to some areas in Africa.

In the exploitation of these soils, as for instance the basaltic acid oxisols of central Brasil, some of which have high potential because of their topography and physical characteristics, the use of leguminous crops in the rotation system is a practice of extremely high value in developing and maintaining of high potential for food production. Soybean culture is a good example and its expansion is helping the farmers and the economy of the region. Possibly the main advantage of the use of leguminous crops is the potential for obtaining free nitrogen from the air. However this advantage will be realized only if the symbiosis of plant vs bacteria can operate effectively. Inoculation of the seeds or the existence of a population of efficient strains in the soil are not perse a guarantee of adequate formation and functioning of the nodules. Several environmental factors acting upon the bacteria, upon the plant or the interaction of environment vs bacteria vs plant may limit or inhibit entirely the attainment of N_2 fixation and thus limit attainment of high yields. What is frequently forgotten by farmers and even agronomists is that the recommendations of fertilizers of soybeans are based on the assumption that a great part of the nitrogen will come through symbiotic fixation. In some regions the soil analysis laboratories recommend only a small amount of nitrogen to function as a starter before fixation begins, and in other regions the amount recommended is nil. So, if the soil is low in combined nitrogen (which is more frequent in the developing countries) and some factors inhibit nodule formation and/or fixation, the response of the plants to the other nutrients will be limited, the yield will be hampered and the investment in the other inputs will be partially or totally wasted.

The neutralization of high acidity and its linked factors, Al and/or Mn toxicity, and correction of any nutritional deficiencies is essential

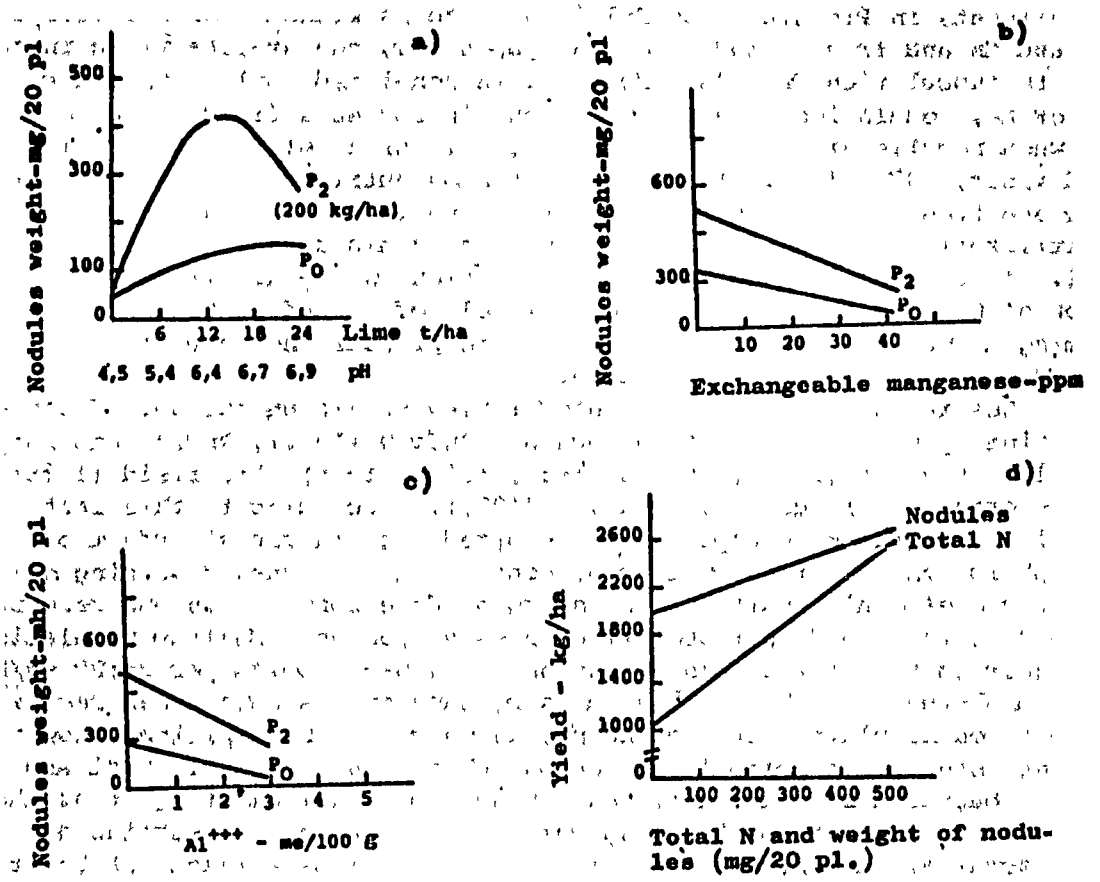

Figure 1. Relationships between:

a) Dry weight of nodules vs. lime at two levels of applied P_2O_5;

b) Dry weight of nodules vs. exchangeable manganese at two levels of applied P_2O_5;

c) Dry weight of nodules vs. exchangeable aluminum at two levels of applied P_2O_5;

d) Grain yield vs dry weight of nodules and total tissue nitrogen.

Data are for soybeans var. Bienville in oxisol, S. Angelo, RS, Brasil. (Vidor, Freire).

to the attainment of high N_2 fixation and high productivity. In earlier experiments in Rio Grande do Sul in acid soils without toxic levels of Al and Mn and free of indigenous *R. japonicum*, the average yield increase due to inoculation was 44%. However, in non-limed soils with toxic Al or Mn, nodulation in experiments and in soybeans fields is absent or when nodules are formed they have little or no efficiency (Freire and Vidor, 1970) (Fig. 1). The more common nutrient deficiency in tropical and sub-tropical regions is phosphorus, and in many areas intensive fertilization has been used for only a few years or has not been used at all. In some soils phosphorus fertilization *per se* alleviates the inhibition of nodulation, but where toxic levels of Al and/or Mn are present liming as well as phosphorus is needed to promote the nodulation necessary for adequate N_2 fixation.

Several authors have presented review papers on the soil factors limiting symbiotic N_2 fixation such as: Andrew (1962), Andrew and Norris (1961). Vincent (1965), Norris (1958, 1964b, 1965), Masefield (1958), Hallsworth (1958), de Mooy *et al.*, (1973). According to this last mentioned review many papers have reported the beneficial effect of phosphorus on number and weight of nodules of soybeans, depending on variety, seasonal conditions and stage of development. In one report there were 2-to 22 - fold increases with these variables. Maximum nodulation is reported to occur at concentrations of 400-500 parts per $2x10^6$ kg/ha of phosphorus. On the other hand, some experiments have shown reducing effects on nodulation due to phosphorus which may be explained, however, by the interaction effects between phosphorus and calcium. Host and nodule bacteria may have different phosphorus requirements just as there are different requirements among varieties and among the strains of the bacteria. A search for strains with optimum performance at lower P supply would be desirable (along with tolerant varieties) and also another approach would be research on placement methods whereby nodulation would be promoted with a minimum of phosphorus fertilizer. In an earlier study involving an out-door pot experiment, de Mooy and Pesek (1966) had reported a highly significant curvilinear response to phosphorus in number, weight and leghaemoglobin content of nodules. The phosphorus vs calcium interaction significantly affected number and weight of nodules and significant differences were found in varietial responses. Demeterio, Ellis and Paulsen (1975) reported that nodule weight and nitrogen fixed were greater for a soybean variety tolerant of low P levels. Zinc deficiency and phosphorus excess may influence nitrogen fixation directly by affecting nodule nutrition and indirectly by affecting host nutrition. In soils of Rio Grande do Sul, Goepfert (1971), and Goepfert and Freire (1972) reported that: 1. phosphorus had a highly significant effect on the stimulation of nodulation and yield; 2. in a soil with Al toxicity phosphorus fertilizer was more important than lime in enhancing nodulation and dry matter of plants; 3. high correlation was found in the interaction P vs yield vs nodule weight. (Fig. 2). In a field experiment now in the third year, Kolling *et al.*, (1976) studied the effects of lime, phosphorus and potassium in relation to the behavior of soybean varieties in an acid oxisol (pH 4.7; Al^{3+}, 1.5 me/100g; P, 6 ppm and K 63 ppm).

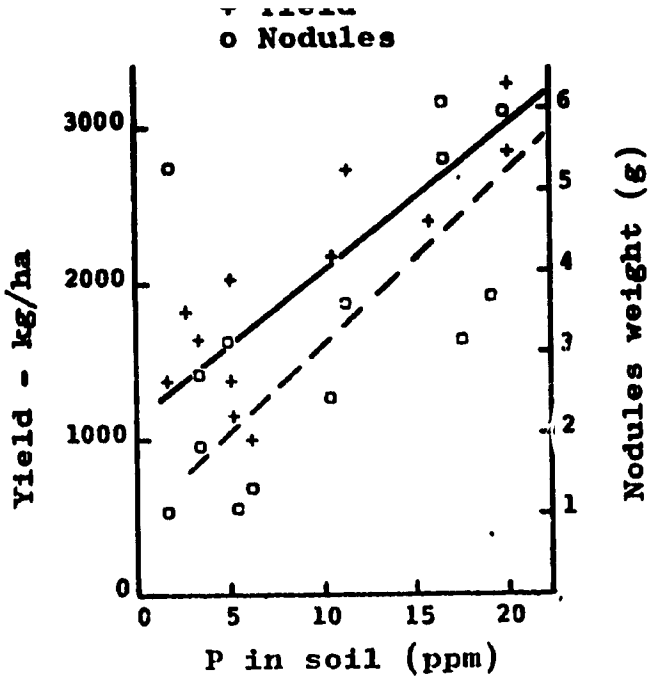

Figure 2. Regressions of available soil phosphorus versus grain yield and nodule dry weight of soybeans. Soil: B. Retiro, RS (Goepfert, C. F.).

Phosphorus fertilizer gave a highly significant response in nodule weight and yield in the three years of the experiment and lime had a less marked effect.

Calcium, pH and Some Linked Factors

Growth and activity of *Rhizobium* as well as effective nodulation have a high requirement for calcium. However, it is difficult to separate the nutritional effects of added Ca and the neutralizing effects of $CaCO_3$. In the review paper of de Mooy *et al.*, (1973), the role of calcium in symbiotic N_2 fixation is well documented as being more important to the bacteria than Mg, Ba, K and H, in this order. Responses to correction of soil acidity were due to increased calcium supply rather than to decreasing H-ion concentration. Maximum yields in acid soils would require a larger calcium supply than in less acid soils. Foy (1974a) pointed out that calcium is required more for root infection and nodule initiation than for nodule development or plant growth. In view of the close association of Ca vs Al vs Cu, varieties selected for greater calcium efficiency may also be more tolerant to excess aluminum and low copper availability in soils.

According also to the review of de Mooy *et al.*, (1973) one of the most important findings during the past decade was that soybeans do not necessarily require high pH for optimum growth. Earlier concepts were that soybeans had an optimum pH range between 6.5 and 7.0. Ohlrogge

reported that the optimum range was pH 5.8 to 6.2. Factors related to soil pH would affect plant and bacteria in a triple interaction.

Hallsworth (1958), Andrew (1962), Vincent (1965), Foy (1974a, 1974b) have also reviewed the indirect and direct effects of soil acidity on nodulation and N_2 fixation. And Norris (1964, 1965) has presented the hypothesis that tropical legumes (including soybean) are ancestors of the family *Leguminosae* and have evolved together with its bacterial symbiont, under the poor fertility, acid soil conditions which predominated in the Cretaceous period. Thus, tropical legumes have developed and maintained a high capacity for extracting nutrients including calcium from the soil, and the associated bacteria during evolution have selected the plant variants which produce alkaline products (which tend to neutralize the acid environment) and these bacteria grow slowly in culture media. On the other hand, temperate legumes have evolved and are more adapted to high fertility non acid soils and their rhizobia, which evolved in these conditions, produce acid and have rapid growth. From a practical standpoint and when one considers the better behavior, growth, nodulation and establishment of the majority of the tropical legumes in comparison with the majority of the temperate legumes in poor acid soils, Norris' theory seems valid. However, it has been subjected to some criticism (Parker, 1968), Munns (personal communication). Norris (1958) expressed the opinion that tropical legumes would not need lime as much as the temperates. His work and that of several others have pointed out that the pH *per se* was not a strong limiting factor on nodulation and symbiotic N_2 fixation by soybeans; however, restrictive effects do exist with the factors sometimes linked to low pH. Munns (personal communication) has reported, on the basis of a field experimental work with a large group of temperate and tropical legumes, that soybeans need a high amount of lime to reach 90 percent of maximum yield but that the relative yield increase was low when compared to other tropical and temperate legumes. With lime there was no increase in nodule number, but weight of nodules, N_2 (C_2H_2)/g and percent nitrogen were increased. He concludes that previous suggestions that the tropical legumes in general have a special tolerance to soil acidity factors may have been based on insufficient sampling of species or soils. Reskasem (personal communication) has confirmed that nodulation by slow growing rhizobia has a relatively high tolerance to soil acidity, but contrary to Norris' suggestion nodulation of soybeans and cowpea strongly responded to lime. He also found the effect of soil acidity on nodulation to be highly dependent on the number of rhizobia in the inoculum. Nodulation of soybean at pH 4.5 responded to lime (pH 6.7) only at levels less than 1,000 rhizobia per seed. At higher levels of inoculum the effect of acidity was overcome by number of bacteria. These results were confirmed by Freire *et al.*, (1974).

Several authors (Norris, 1958, 1964; Andrew and Norris, 1961) have also indicated that no sharp frontier exists between the groups in relation to effect of lime on nodulation. The work of Munns (personal communication) has indicated also that within each group there is a great diversity in the response to lime. The same lack of uniformity exists in the

varieties of the genus *Glycine max* (Cabeda & Freire, 1968; Coutinho *et al.*, 1971).

Rorison (1958) and Rorison *et al.*, (1958) have reviewed the extensive literature on the effect aluminum and manganese toxicities on plants. Foy (personal communication), Foy (1974a, 1974b) and Foy *et al.*, (1969) reported that aluminum appears to interfere to different degrees with the uptake and use of calcium, and that aluminum tolerance in soybean varieties seems to be closely associated with the ability to absorb and transport of calcium in the presence of excess aluminum. The inhibitory effect of aluminum on the calcium absorption is well confirmed as is the importance of the Ca:Mn ratio in the plant tissue rather than the content of Mn alone in determining the tolerance to or severity of manganese toxicity in plants. These observations explain the reported effect of aluminum in enhancing the severity of the Mn toxicity symptons. However there were no previous reports showing increased uptake of manganese to be associated with increased aluminum levels (Cabeda and Freire, 1968) with detrimental effects on dry matter yield, nodulation and total nitrogen in the plants. Coutinho *et al.*, (1971) observed the importance of the Ca:Mn ratio to nodulation and tolerance; varieties with low tolerance to toxicity showed higher responses to lime and had higher calcium absorption than non-tolerant varieties.

In another study Foy (1974a) laments the existence of very little information on the direct effect of aluminum on rhizobia. One must distinguish between the effect of aluminum on free rhizobia cells in the soil, on the process of nodule formation and on N_2 fixation in the nodules. Kamprath and Foy (1971) studied the effects of soil acidity and calcium concentration on N_2 fixation, considering not only the growth of the legume but also the growth of the bacteria. They claim that: 1. the main effect of aluminum on N_2 fixation is probably related to its detrimental effect on root growth and consequently fewer nodules; 2. the interference with calcium uptake and transport is related with nodule initiation which has a much higher calcium requirement than either nodule development or growth of the plant; 3. plant varieties within species differ markedly in aluminum tolerance; 4. considerable evidence exists that aluminum tolerance is correlated with the ability of a plant to absorb and utilize phosphorus in the presence of aluminum; 5. temperate legumes are more sensitive to manganese toxicity than the tropicals and calcium uptake is related to manganese toxicity in that it reduces manganese transport to the plant tops. Sartain and Kamprath (1975) observed that: 1. the effect of liming was related to percent aluminum saturation of the ration exchange sites, and the toxicity affected growth of tops, roots and nodules and also nutrient accumulation of soybeans; 2. increase in nodule number was significantly correlated with the calcium content of the primary root, with the total phosphorus and magnesium contents of the nodules and with the Ca/Al ratio of the nodule; 3. with high Al saturation a small number of large, apparently inefficient nodules develop, whereas under low Al saturation a large number of small, more effective nodules are formed; 4. little difference was observed in weight of nodules between treatments because

of the high mass/nodule ratio of large nodules formed under acidic conditions.

Cabeda and Freire (1968) studied the effects of Al and Mn toxicities in nutrient solution on soybeans, avoiding by a special technique the detrimental effects of the Al on the absorption of other nutrients such as phosphorus and calcium. The severity of Mn toxocity symptons was in direct relation to the Al content of the solution and Mn absorption was also related to the Al content. Under the conditions of the experiment, nodulation was not greatly affected. In another study in the greenhouse, Cabeda and Freire (1968) used four *R. japonicum* strains, three levels of lime, two soybean varieties (one tolerant to acid soils) and three soils. The following conclusions were reached: 1. the effect of lime on nodulation was more marked in the soils with more Al; 2. number and weight of nodules were affected as much as nitrogen in the plants tissue which would indicate that the effect was on the formation and functioning of the nodules; 3. number and weight of nodules were less affected in the soil high in Mn and low in Al than in the other two soils (high in Al and low in Mn); 4. the tolerant variety absorbed more calcium and less manganese than the non-tolerant variety and had better nodulation in terms of number and weight of nodules; 5. as in the nutrient solution experiment there was a tendency for the Al to enhance the absorption of manganese.

Franco and Dobereiner (1971) in a greenhouse experiment with a high exchangeable manganese soil reported: 1. liming increased N_2 fixation and plant growth 2-3 times; 2. number, weight and size of the nodules were highly affected by Mn toxicity; 3. symbiosis on the soybean variety that absorbed more Mn was more affected by lime than on the other variety. Vidor and Freire (1971, 1972) reported the following conclusions from a field experiment in an oxisol high in Al and Mn: 1. nodulation increased with phosphorus levels; 2. highest dry weight of nodules occurred with 6 tons/ha of lime and 400 kg/ha P_2O_5; 3. there was very little influence on nodulation when either lime or phosphorus was used alone; 4. without lime, the yield was doubled in response to phosphorus application but no increase in nodule weight was observed, which could be explained by the utilization of more soil nitrogen. In another experiment (Vidor *et al.*, 1974) in an acid soil high in Al and Mn, there were significant effects of lime, phosphorus and of the interaction lime vs phosphorus and of the interaction lime vs phosphorus on nodule weight and yield. In a field experiment now in the third year with seven *R. japonicum* strains and two lime levels in an acid soil (pH 5.1) low in Al and Mn (Freire *et al.*, 1976) the following tentative conclusions have been reached: 1. no significant effect was observed on the N_2 (C_2H_2) fixation per gram of nodules and per plant by the lime application but the interaction lime vs strains was significant at least on two sampling dates; 2. weight of nodules was affected significantly by lime and by the interaction lime vs strains, but at only one of the three sampling dates.

De Mooy *et al.*, (1973) reviewed the literature on the effect of potassium on nodulation of soybeans and report that they are as variable

as those with phosphorus. The effects of potassium are commonly beneficial but they are generally linked with the level of the available phosphorus and also with lime ($CaCO_3$). One experiment is cited in which maximum nodulation required a potassium application of 600 to 800 ppm of K. A detrimental effect is frequently reported at high levels of application. Kolling *et al.*, (1976) found that the variety Hardee was highly tolerant to potassium defficiency and the contrary occurred with varieties Bragg and Planalto. Nodulation of these varieties on K deficient plots was related to their tolerance. (Table 5)

Borkert (1973) observed that KCl without lime increased manganese availability in the soil and the Mn concentration in the plant tissue. In a pot study there was a decrease in nodulation and yield with added K, but in a field experiment no such effect was observed. The detrimental effect of KCl is attributed by other workers to Cl. In a field study with alfalfa, KCl without lime reduced yield and nodulation. And Freire *et al.*, (1969), Freire and Vidor (1970) reported reduction of nodulation in a field experiment due to application of KCl fertilizer without liming but no damage with liming.

The beneficial effects of molybdenum on symbiotic N_2 fixation is well documented by de Mooy *et al.*, (1973). Results commonly are positive

Table 5. Yield (kg/ha) and Nodules (g/10 pl.) of soybeans vs P, K, lime, 1976 (4th years). Mean 9 rep. (Kolling *et al.*)

Treat*	Varieties					
	Planalto		Hardee		Bragg	
	Yield	Nod.	Yield	Nod.	Yield	Nod.
PO	673	0,7	988	1,2	346	0,9
P1	1982	2,0	1587	2,1	1656	2,9
P2	1842	2,3	1768	2,4	2042	3,9
KO	1084	1,3	1092	1,7	1051	1,9
K1	1449	1,5	1548	2,1	1779	2,7
K2	1965	2,2	1704	1,8	1713	3,1
Ca o	1065	1,8	1180	2,0	977	2,7
Ca 1	1599	1,5	1417	1,9	1608	2,5
Ca 2	1834	1,6	1745	1,9	1958	2,6

	kg/ha/year	kg/ha/year	kg/ha(1st year)
* Treatments:	P1-100	K1 - 50 Ca1 (lime)	- 3,000
	P2-200	K2 -100 Ca2 (lime)	- 6,000

from Mo application either by seed treatment, soil treatment or foliar application, when the soil has a low pH but no toxic levels of exchangeable Al and/or Mn. Where the soil is limed or has a high pH the availability of molybdenum is usually high and there are no responses to applications. According to Burton and Curley (1966) molybdenum compounds applied to the seed are harmful to bacterial survival in the inoculant if not applied just prior to planting.

Sulphur is another important element because of its role in protein synthesis, and deficiencies have been reported in soils of central Brasil. Borges *et al.*, (1974) reported the following conclusions from a greenhouse pot experiment: 1. lime was the variable with most influence on number and weight of nodules; sulphur was second, molybdenum had no effect; 2. 20 ppm of S increased nodule weight without lime by 74 percent and with lime by 26 percent.

N_2 fixation is reduced by water stress or waterlogging. Pankhurst and Sprent (1975) found that N_2 (C_2H_2) fixation is reduced and respiratory activity of nodules declines when subjected to water stress, but the actual activity of the bacteroid tissue is relatively unaffected. Shortage of water is likely to be a major cause of nodulation failure apparently even under tropical conditions, but the effect is likely to be mainly on rhizobial growth and survival, and to be greater under conditions of alternate wetting and drying and low pH (Vincent, 1965).

Brose (unpublished information) obtained highest N_2 (C_2H_2) fixation with soil moisture at 75% of the field capacity; at 50% for fixation was extremely reduced and the same occurred without light for 24 hours. Soil moisture was reduced to the treatment levels 5 days before measurements of the fixation. The influence of soil moisture, as light, was by the reduction of the supply of photosynthates. (Fig. 3).

Effects of Pesticides

The effects of pesticides on rhizobia and on the nitrogen fixing process have been extensively reviewed by Smith (personal communication). Although many investigations have been carried out with insecticides, little is known on their effects on rhizobia and nodulation. Harmful effects are reported in laboratory studies but not in the field, at least for the most common insecticides.

The incidence of insect pests on soybeans in the tropics seems to be higher than in the United States and the massive use of insecticides is becoming a problem. The investigations on the effects of these chemicals should probably have higher priority than at present. In earlier studies in soybeans in Brasil, Myiasaka and Silva (1956) recommended the use of Arasan in spite of a slight inhibitory effect on nodulation. Ruschel and Costa (1966) reported harmful effects on nodulation from mercurials but not from other fungicides and insecticides studied. Galli (1959) reported no damage from the insecticides and fungicides except for one insecticide when applied at the recommended rates.

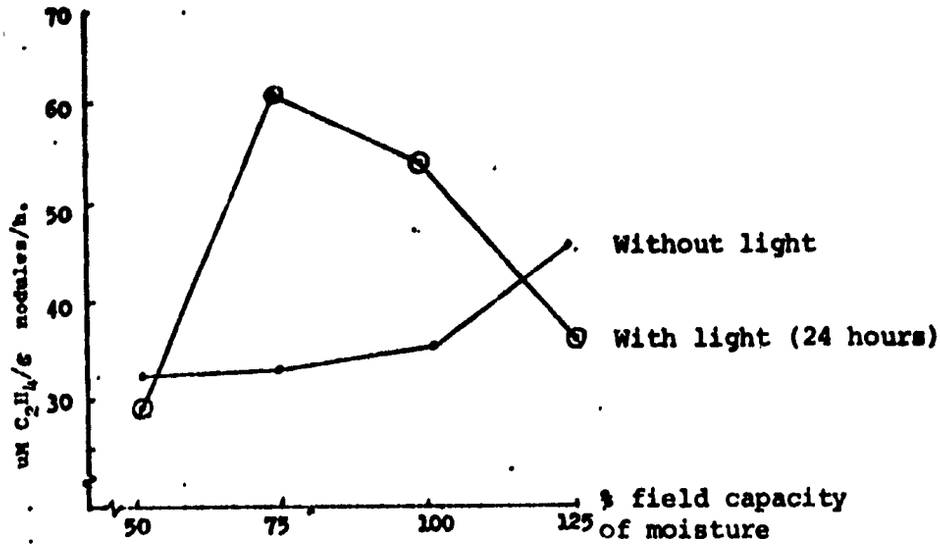

Figure 3. Effect of soil moisture on the specific nodular activity in $N_2(C_2H_2)$ fixation of soybean, after 5 days at the treatment levels, with and without light (25 h.) in a greenhouse experiment. (Brose, Mielniczuk, Freire).

According to the review of Smith (personal communication) considerably more literature is available on the influence of herbicides on rhizobia and on the legume-rhizobia relationship. There is indication that one of the soil processes most likely to be affected by the application of herbicides is legume nodulation. From the reviewed literature the following conclusions are drawn: 1. bacterial sensitivity in the laboratory (in vitro) depends on the rhizobia species, on the strains and on the dosage of the herbicide; 2. in greenhouse or pot experiments some investigators have found harmful effects and others not. 3. tap root nodulation is more sensitive than lateral root nodulation; 4. in field use herbicides may be detrimental if applied at rates in excess of those recommended by the manufacturers, if applied carelessly or if there are cumulative residual effects; 5. the evidence with herbicides indicates that, to a certain extent, decrease in nodulation is brought about by the injurious effect on plant vigor and root growth and not by a direct effect upon the bacteria. In a recent study, Lorenzi and Araujo (1974) reported the following results from a soybean field trial utilizing herbicides at the recommended rates: 1. the herbicides DCPA, fluorodifen, trifluralin and linuron reduced number of the nodules produced by the native bacteria in the non-inoculated plots; 2. only linuron (4kg/ha) reduced significantly the percent nitrogen in the plant tops.

Concerning seed treatment with fungicides Smith (personal communication) reports conflicting results from the investigators, due probably to variance in the experimental methods. In a way there is concordance

on the detrimental effect observed when both fungicide and inoculant are applied on the seed, and the magnitude of this effect is in direct relation with the time of exposure. Pendleton and Hartwig (1973) conclude that: 1. high quality seeds do not ordinarily require fungicide seed treatment because small differences in stand generally do not influence seed yields; 2. weathered, poor quality seed may respond with higher yield, but then the treatment must be done several weeks ahead of planting and inoculation just prior to planting; 3. where soybeans are planted for the first time seed treatment is not recommended because inoculation contributes more to yield than the fungicidal treatment. It would be advisable then to increase rate of seeding.

ESTIMATION AND MEASUREMENT OF SYMBIOTIC FIXATION

Evaluation of nodulation is important to agronomists in surveying soybeans fields and to researchers in estimating the adequacy of the nitrogen supply to the plants. Lack of this evaluation may lead the researchers into gross errors in interpretation of the results of experimental work:

1. In experiments involving competition of lines and cultivars, differences in nodulation and N_2 fixation between the materials under tests may lead to higher yield of those with higher nitrogen supply from the symbiosis due to the different interactions of plant genotypes vs strain in the inoculant and/or soil population.
2. Another misleading situation occurs when a bad inoculant is used or when there are nutrient deficiencies, toxic factors, high levels of combined nitrogen or combinations of factors which inhibit nodulation and/or N_2 fixation so that plants will be then reacting only to soil nitrogen; the selected genotypes transported to other locations and subjected to inoculation or a native or naturalized rhizobia population may react completely differently than in the breeding nursery.
3. Differences in plant genotypes at a breeding nursey with a certain *R. japonicum* population may be completely altered when the same genotypes are tried or the selected cultivars are released and planted at different locations with different rhizobial populations.
4. In fertility experiments lack of adequate inoculation or lack of establishment of an efficient symbiosis resulting in an inadequate nitrogen supply will limit response of the plants to other treatments; lack of evaluation of the nodulation may lead the worker to an erroneous conclusion about the lack of response to tested treatments. It is evident that if the soil is rich in combined nitrogen or if nitrogen fertilizer has been applied, the plants will respond to the treatment; however, there is the possibility that this type of response will be different from the possible response where mineral nitrogen is low and symbiotic nitrogen would

be otherwise available.

5. In fertility or other types of experiments, lack of adequate nodulation and N_2 fixation may be due to the selection of an inadequate variety with little or no affinity to the inoculum or to the indigenous *R. japonicum* population; the expected responses to the treatments under test may then be hampered by the limited nitrogen supply.
6. The precision of the evaluation of the nodulation depends on the type of experiment and to the importance attributed to N_2 fixation in relation to the treatments under test; the methods for evaluating the various parameters involved in N_2 fixation include: (a) grading of the nodulation in visual categories as to number, weight, position and internal color of the nodules; (b) determining nitrogenase activity by the acetylene reduction technique; (c) determining total plant nitrogen by Kjeldahl analysis; (d) using labeled nitrogen for tracing; (e) doing serological identification of strains responsible for the nodules.

CONCLUSIONS AND RECOMMENDATIONS

1. Selection of efficient *R. japonicum* strains among the existing population, comparing introduced and adapted naturalized strains, is the practical and feasible way of having good inoculants. Other characteristics such as behavior under different environmental conditions, competitive and survival abilities and range of varietal affinity must also be investigated to achieve increased reliability of the inoculants.
2. The worldwide actual and future importance of soybeans would justify a specialized international center for culture collection and preliminary greenhouse strain selection.
3. At some areas in which soybean is developing, as in Brasil, the institutions now working on strain selection should standardise the testing methods and the requirements for strains releasing and recommendation.
4. Nodulation characteristics such as weight, position, color, size and number of nodules are of value in the evaluation of the fixation efficiency of the strains.
5. A complex relationship exists between soybeans, *R. japonicum* and the environment which governs the nodulation and N_2 fixation. All three factors must be investigated to the attainment of high N_2 fixation and high yield.
6. Research on the composition of inoculants, their performance at different environmental conditions, methods of application, inoculum rate, etc, must be intensified at regional levels.
7. The problem of introducing better strains into old producing areas deserves more research, which should take the three different approaches: through the plant, the bacteria and/or the environment. Some success seems to be reached through the application of ultra-

high massive inoculation; however under large scale field plantings this method would probably be impractical.

8. Many soil factors influence rhizobial behavior in tropical and sub-tropical areas hampering the nodulation in first plantings or low population soils. The main ones seem to be: high soil temperature, low moisture and toxic levels of aluminum and manganese. Massive inoculation seems to have enhanced nodulation and yield but more research is necessary for more precise information on types of inoculants and methods of application.
9. Contribution of N_2 fixation to soybean growth depends on many factors related to the plant, the bacteria and the environmental conditions which must be satisfied for maximum contribution. With normal good field conditions N_2 seems to be the second source of nitrogen to soybeans, mineral nitrogen being the first from the soil. However, considering the high nitrogen requirement of the soybean plant, the N_2 fixation has contributed on a large scale.
10. Soybean genotypes vary markedly in their affinity for *R. japonicum* genotypes. For the selection and breeding of soybeans consideration must always be given to the symbiotic capacity of the material under testing. *R. japonicum* populations at the nursery fields and at the areas to be covered by the released varieties must be surveyed and tested.
11. Nitrogen fertilizer applications have given significant responses only when nodulation and/or N_2 fixation are hampered by some limiting factor. To prevent reduction in yields in first plantings, the application of nitrogen would be advisable if after planting failure of nodulation is apparent.
12. Availability of photosynthate seems to be the major factor responsible for the limited N_2 fixation, lack of response to more mineral nitrogen and for the soybeans yield plateau. Research to increase this level would envolve a search for high photosynthetic capacity, reduced photorespiration and high symbiotic capacity. Under normal field conditions, this factor is also responsible for the decline in the N_2 fixation at end of flowering or early pod filling and thus limiting yield, if soil nitrogen is in short supply.
13. In many areas where soybeans are being established nutritional and toxicity factors related to soil acidity are presently important factors in the limitation of N_2 fixation. The identification and adequate control of these factors are important to increase yields.
14. In less developed regions varieties can be developed with tolerance to low nutrient availabilities and toxicities. N_2 fixation and yield can be higher than with those varieties developed for freedom from adverse environmental conditions.
15. In any experimental work or with a field cropped to soybeans (or any symbiotic legume) it is essential to have an adequate evaluation of the nodulation and/or N_2 fixation in order to estimate the adequacy of the inoculation and supply of nitrogen through the symbiosis and help the adequate interpretation of the results.

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ASSOCIATIONS OF SOME UNCOMMON LEGUMINOUS PLANTS WITH VARIOUS RHIZOBIA

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INTRODUCTION

Leguminous plants and their symbiotic partners, the root nodule bacteria, work as a team. The full potential of a leguminous plant can be measured only when the proper rhizobia are provided for effective nodulation, so that the plant can utilize the vast reservoir of free nitrogen (N_2) in air. Yet agronomists often collect germplasm of the host plant without remembering to also collect nodules or soil from around the roots as a possible source of the proper nodule bacteria. When this occurs, the challenge to the rhizobiologist is to find effective bacteria for each leguminous species. If the nodulating traits of the plant species or genus (cross-inoculation group) are known, the problem is less complicated because the first essential of a symbiotic association is nodulation. The search can therefore be restricted to species or varieties of *Rhizobium* capable of producing nodules on plants in the particular cross-inoculation group. On the other hand, when the cross-inoculation group of the leguminous species is not known, or it is not known whether or not the leguminous host is nodulated by any rhizobia, the problem becomes more difficult.

The cross-inoculation grouping of a leguminous species in question is determined by planting surface sterilized seeds in several jars or pots containing a sterile substrate and a suitable nitrogen-free nutrient solution and inoculating plants in different jars with a wide range of species or varieties of rhizobia and noting nodulation after a 3 to 5 week growth period. After the cross-inoculation grouping has been established, the next step is to carry out similar tests with a wide range of *Rhizobium* strains capable of nodulating the particular host and measuring N_2 fixation visually, by chemical analysis, or by acetylene reduction.

The objectives of this study were: 1) to study the nodulating characteristics of various leguminous species imported to the U.S. for evaluation as food or forage legumes and 2) if possible, prepare an effective *Rhizobium* inoculum for each plant species.

EXPERIMENTATION

The scarcity of seeds of many of the new plant introductions determines the choice of method of plant culture, as well as the number of inoculation treatments. Test tubes (25 x 150mm) are very satisfactory for small-seeded legumes, but Leonard jars (32 ounce Boston rounds) or one-half gallon glazed jars are preferred for the larger seeded legumes. Leonard jars were used exclusively in these tests. The number of replicates varied with the supply of available healthy seedlings. Seeds were surface sterilized by immersing in 70% ethyl alcohol for 5 minutes and 3% calcium hypochlorite solution for 20 minutes. After washing several times with sterile distilled water, seeds were placed on moist blotter paper in 200 mm petri dishes for germination.

In making the inocula, two to five strains of rhizobia isolated from and known to be effective on their parent host were grown and incorporated into a peat carrier medium. These were given the genus name of the parent host. The one exception was the composite inoculum for the cowpea cross-inoculation group which was designated "EL". The inoculum contained four strains of rhizobia--one for *Crotalaria* sp., *Desmodium, Stylosanthes, and Vigna*. This treatment was included because several of the plant species were known to be in the cowpea cross-inoculation group.

After a period of 6 to 7 weeks in the growth chamber in a nitrogen-free substrate, the plants were compared with non-inoculated control plants for size, color and vigor. The plants were then harvested and roots were examined for nodulation.

RESULTS

The results are summarized in Table 1.

Crotalaria, Desmodium, Dolichos, Galactia, and *Indigofera* species responded effectively to a wide range of rhizobia. *Canavalia* species and two species of *Phaseolus, P. lunatus,* and *P. acutifolius* (tepary bean) gave effective responses to the same strains of rhizobia. These *Canavalia* and *Phaseolus* species were nodulated by strains of rhizobia effective on other plants in the cowpea cross-inoculation group - *Arachis* sp., *Vigna* sp., *Crotalaria* sp., and *Desmodium* sp., but these strains were of no benefit to these hosts. Likewise, rhizobia isolated from and effective on jackbean, lima bean, and tepary bean were not effective on cowpea, peanut and tick clover.

The *Sutherlandia* species, and *Swainsona salsula* were nodulated by a broad spectrum of rhizobia, but none of these associations brought about N_2 fixation.

Cercidium floridum and *C. microphyllum* were not nodulated by any of 13 inocula prepared with effective strains of rhizobia isolated from different genera of leguminous plants. *Cercidium* species might

possibly be nodulated by other rhizobia - or this genus could be one that does not nodulate. This would have to be based on more extensive tests.

Exploratory tests as described here constitute a necessary preliminary stage in developing an effective inoculum. When plants of a single inoculation treatment show a wide variation in response, consideration should be given to isolation of rhizobia from the nodules which appear to be the most effective and then testing these for N_2 fixing ability in a subsequent test. If the plants in a single inoculation treatment show uniformly good N_2 fixation, then the second stage of development should be to test a large number of rhizobial strains isolated from species within that particular genus. This should enable selection of the most efficient strains for further testing under the prevailing field conditions.

Table 1. Reactions of some uncommon leguminous plants to inoculation with various rhizobia.

Host Plant	Effective rhizobia from ———	Produced nodules	Fixed N_2
Canavalia ensiformis	*Phaseolus lunatus*	Yes	Very effective
Canavalia lineata	*Canavalia ensiformis*	Yes	Very effective
	Vigna unguiculata	Yes	No
	Arachis hypogaea	Yes	No
Centrosema virginianum	*Centrosema* sp.	Yes	Very effective
	Galactia sp.	Yes	Very effective
	Vigna unguiculata	Yes	No
	Arachis hypogaea	Yes	No
Cercidium floridum	Rhizobia from 13		
Cercidium microphyllum	different genera	No	No
Crotalaria trifoliastrum	*Crotolaria* sp.	Yes	Effective
	Vigna sp.	Yes	Effective
	Arachis hypogaea	Yes	Effective
	Desmodium sp.	Yes	Effective
Desmodium cuspidatum	*Desmodium* sp.	Yes	Effective
Desmodium distortum	*Stylosanthes* sp.	Yes	Effective
Desmodium neomexicanum	*Crotolaria* sp.	Yes	Effective
Desmodium ovalifolium			
Desmanthus velutinus	*Crotalaria* sp.	Yes	No
Desmanthus vergatus	*Desmodium* sp.	Yes	No
	Erythrina sp.	Yes	No
	Stylosanthes sp.	Yes	No
	Desmanthus sp.	Yes	Very effective

Table 1 *continued.*

Host plant	Effective rhizobia from --	Produced nodules	Fixed N_2
Dolichos biflorus	*Dolichos* sp.	Yes	Effective
Dolichos uniflorus	*Desmodium* sp.	Yes	Effective
	Crotalaria sp.	Yes	Effective
	Vigna sp.	Yes	Effective
	Arachis hypogaea	Yes	Effective
	Astragalus sp.	Yes	Effective
	Robinia sp.	Yes	Effective
Galactia jussiaeana	*Galactia* sp.	Yes	Very effective
	Robinia sp.	Yes	Very effective
	Crotolaria sp.	Yes	Slight
	Stylosanthes sp.	Yes	Slight
	Vigna sp.	Yes	Slight
Indigofera arrecta	*Indigofera* sp.	Yes	Effective
Indigofera endecephylla	*Centrosema* sp.	Yes	Effective
	Galactia sp.	Yes	Effective
	Desmodium sp.	Yes	Effective
	Clitoria sp.	Yes	No
Indigofera glandulosa	*Indigofera* sp.	Yes	Effective
Indigofera rautanenii	Composite 'EL'	Yes	Effective
Indigofera sumatrona			
Indigofera teysmanii			
Indigofera subulata	*Indigofera* sp.	Yes	No
	Composite 'EL'	Yes	Moderate
Phaseolus lunatus	*Phaseolus lunatus*	Yes	Very effective
Phaseolus acutifolius	*Canavalia ensiformis*	Yes	Very effective
	Composite 'EL'	Yes	No
Psophocarpus tetragonolobus	*Psophocarpus* sp.	Yes	Moderate
Psophocarpus palustris	*Erythrina* sp.	Yes	Effective
	Crotolaria sp.	Yes	Effective
	Stylosanthes sp.	Yes	Moderate
Sutherlandia fructecosa	*Astragalus* sp.	Yes	No
Sutherlandia microphylla	*Baptisia* sp.	Yes	No
	Centrosema sp.	Yes	No
	Clitoria sp.	Yes	No
	Desmodium sp.	Yes	No
	Dolichos sp.	Yes	No
	Galactia sp.	Yes	No
	Robinia sp.	Yes	No
	Tephrosia sp.	Yes	No
Swainsona salsula	*Astragalus* sp.	Yes	No
	Baptisia sp.	Yes	No
	Centrosema sp.	Yes	No
	Galactia sp.	Yes	No
	Indigofera sp.	Yes	No
	Robinia sp.	Yes	No
Teramnus labialis	*Robinia* sp.	Yes	Very effective
	Crotalaria sp.	Yes	Effective
	Stylosanthes sp.	Yes	Effective
	Desmodium sp.	Yes	Effective

EFFECTS OF SOIL ANTAGONISTS ON SYMBIOSIS

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INTRODUCTION

The prestige of legumes in practical agriculture lies in their ability to symbiose with the root nodule bacteria belonging to the genus *Rhizobium* and to become independant of soil nitrogen. Such symbiosis has been profitably exploited in the temperate zones of the world through concentrated and devoted research, improvement of pastures (and thereby animal production) and development of unproductive soils into suitable agricultural lands being the most spectacular achievements along with the improvement of legume grain yields.

The root-nodule bacteria of the legumes are soil inhabitants. They may be indigenous, along with the appropriate hosts, or they may be introduced with a new host by seed inoculation or natural contamination. Whether they are indigenous or introduced, they have to survive and proliferate in the soil along with the millions of soil inhabitants. Being saprophytic in food habit, they have to share the common pool of energy substrates as well as other nutrients with the soil organisms. Although spore formation in rhizobia has been claimed (Bisset, 1952), there is no evidence of a resting phase in the life cycle of rhizobia (Graham *et al.*, 1963). As a result the rhizobia have no special protection against adverse conditions, and they face all kinds of interactions including antagonism by other soil microorganisms. We may find situations where the rhizobia can easily colonize a soil when introduced or where they can not establish themselves even in the rhizosphere of appropriate hosts. Our present knowledge on the effect of antagonists upon legume-*Rhizobium* symbiosis is meagre, although a limited amount of information is available with respect to the survival and persistence of rhizobia in the soil.

RHIZOBIAL NUMBERS AND ROOT INFECTION

The prerequisite for effective symbiosis is the infection of the legume root and nodule formation by appropriate rhizobia. Theoretically one viable cell of the bacteria should be sufficient to form a nodule but several hundred to several thousand viable rhizobial cells per seed may be required at the time of inoculation under field conditions for effective nodulation (Vincent 1970). Using different levels of *Rhizobium*

japonicum cells in soil and sand cultures, Weaver and Frederick (1972) observed that the nodulation of tap roots of soybean was increased with increasing levels of inoculum and the maximum nitrogen fixation was achieved at inoculation levels of 2,000 and 10,000 cells per seed for soil and sand-grown plants, respectively. In presence of a high number of ineffective rhizobia in soil, inoculation with 10^5 to 10^6 cells per seed is needed for effective nodulation (Ireland and Vincent, 1968).

Rhizobium multiplies rapidly in the legume rhizosphere and the stimulating rhizosphere effects may occur as far as 10 - 20 mm from the root surface (Rivira, 1961). In general, the proliferation of a particular *Rhizobium* occurs rapidly in rhizosphere of its host (Purchase and Nutman, 1957; Tuzimura *et al.*, 1964) but stimulation of growth has also been recorded in the rhizosphere of leguminous plants other than the host (Tuzimura *et al.*, 1963) as well as in that of non-legumes (Tuzimura *et al.*, 1964; Chatel and Greenwood, 1973).

NODULATION PROBLEMS DUE TO MICROBIAL ANTAGONISM

Although seed inoculation is the most satisfactory practice of introducing effective strains of *Rhizobium* in a soil for successful symbiosis in legumes, failure of nodulation, despite inoculation, has been recorded. There may be three kinds of problems: (1) the introduced rhizobia fail to colonize the host rhizosphere and to nodulate the host; (2) the inoculant strain(s) may colonize the host rhizosphere to some extend and produce good nodulation during the season of inoculation, but fail to colonize the soil; and (3) the introduced strain can not successfully compete with indigenous rhizobia to nodulate the host and, thus, to exert maximum symbiotic benefit.

Failure of the introduced rhizobia to multiply in the soil or rhizosphere may arise due to soil physical, chemical and nutritional factors, e.g. temperature, desiccation, pH etc., a detailed review on which has been made by Vincent (1965). In addition to these factors; microbial antagonism may also contribute, at least in part, to the failure of legume establishment due to unsuccessful nodulation as may be encountered in problems (1) and (2).

Microbial antagonism leading to unsuccessful nodulation of inoculated legumes has been experienced in Australia (Hely, *et al.*, 1957; Parker 1962; Holland and Parker, 1962), Canada (Webster *et al.*, 1967), South Africa (Hattingh and Louw, 1966a), New Zealand (Greenwood, 1964) and India (Vyas and Prasad, 1960). Partial sterilization by steaming or fumigation of soil have often improved the yield of the legumes (Cass-Smith and Holland, 1958; Beggs, 1964; Khan, *et al.*, 1968), possibly through the control of soil antagonists or their products. Ageing the newly cleared soil, where nodulation problem was evident, by bare fallowing twelve months before sowing (Cass-Smith and Holland, 1958) or inoculating with a high number of rhizobia (Parker, 1962) has also resulted in good nodulation of inoculated clovers.

The second kind of problem where inoculated clovers yielded successful nodulation in the year of sowing but failed to nodulate in

the succeeding years, a problem termed "second year clover mortality", was reported from Western Australia only (Parker, 1962; Marshall, Mulcahy and Chowdhury, 1963). The problem arose mainly due to failure of the rhizobia to colonize the soil as a result of desiccation and high summer temperature (Marshall, 1964; Chowdhury, 1965; Chatel and Parker, 1973) and poor "saphrophytic competence" of the inoculant *Rhizobium trifolii* strain TA1 (Chatel *et al.*, 1968) as well as to toxic factor(s) or antibiosis (Chowdhury, 1965; Chatel and Parker, 1972). T This problem has been overcome by using inoculant strain WU290 in place of commercial strain TA1 (Chatel *et al.*, 1973).

The third kind of problem is entirely microbiological and arises due to competition among rhizobial strains for nodulation sites.

BIOTIC EFFECTS ON *RHIZOBIUM*

The subterranean population exerts neutral, harmful or stimulatory effects upon one another. Some of these effects are categorically spectacular, some may escape our attention unless a specific problem arises. The root nodule bacteria native to a soil, as a part of the complex soil coenoses, or introduced to a soil as an "alien" are also affected by microbial interactions.

Various aspects of microbial interactions have been reviewed by a number of authors namely, Alexander (1964), Baker (1968), Clark (1965, 1967), Jackson (1965), Malcolm (1966) and Park (1960, 1967) among others. Malcolm (1966) proposed as many as ten categories of biological interactions, namely, neutralism, mutualism, commensalism, protocooperation, competition, amensalism, predation, parasitism and three other unnamed interactions. Although it is likely that all these interactions may be operative at any one time in a soil with the complex biocommunities which are further modified by the rhizosphere effects of living roots, little is known regarding the effects of each interaction on the root-nodule bacteria in soil and their activities. For the sake of convenience, I would like to simplify these interactions into three general groups, namely, (a) neutral associative effects (b) beneficial associative effect and (c) harmful or antagonistic associative effects. Although the major theme of this article is to discuss the effects of soil antagonists on symbiosis, a brief mention is made on the neutral and beneficial effects of soil microorganisms on rhizobia.

NEUTRAL ASSOCIATIVE EFFECTS

Many organisms, mainly bacteria have no effect on rhizobia. Some organisms, found inhibitory to the growth of rhizobia in artificial media, had no or little adverse effect in soil suggesting that soil has a protective action against the injurious effect on rhizobia by other microorganisms (Fred *et al.*, 1932). Bhalla and Sen (1973) found that 60 percent of the bacteria isolated from the rhizosphere of uninoculated gram (*Cicer arietinum*) were without any stimulating or inhibitory effect

on the root nodule bacteria of gram, the corresponding figure from inoculated plants being only 34 percent. Hattingh and Louw (1964) also found that majority of the 246 isolates from the rhizoplane and rhizosphere organisms of clover and from the non-rhizosphere soil were without any effect on *R. trifolii*.

BENEFICIAL ASSOCIATIVE EFFECTS

Fred *et al.*, (1932) reviewed a number of earlier works dealing with the stimulatory effects of some soil microorganisms, both bacteria and fungi, upon the growth of rhizobia in artificial media. In recent years, Bhalla and Sen (1973) reported that 50 percent of the rhizosphere bacteria requiring yeast extract were found to be stimulatory to the nodule bacteria of gram. Hattingh and Louw (1966b) found mostly gram-ne negative non=spore forming bacteria from rhizosphere of clover to be stimulatory to *R. trifolii*. Inoculation of rhizobia in association with other isolates onto the seed of the host legume is reported to exert a beneficial effect on the yield and nitrogen content of the host. These beneficial organisms are diverse, for example, *Pseudomonas* and *Achromobacter* spp. (Krasilnikov and Korenyako, 1944), *Bacillus mycoides* (Efron and Milova, 1941) and certain *Streptomyces* spp. (Robison, 1945). Harris (1953) reported that some soil organisms stimulated the virulence of a weakly invasive strain of *R. trifolii*. In the course of investigating the effect of certain root-surface fungi upon the root-hair infection of *Trifolium glomeratum* by rhizobia, Lim (1961) observed that certain fungi (e.g. *Gliocladium, Paecilomyces* and *Humicula)* increased the infection and hastened nodulation. It is not known whether the better symbiotic performance of rhizobia on the host is due to growth stimulation of *Rhizobium* in the rhizosphere or to the effect of probiotic(s) in stimulating the nodulation process. Vincent (1954b) emphasized that "the general significance of the phenomenon and the practicability of making use of such (stimulatory) organisms are matters meriting further investigations." Most recently, Dr. M.J. Trinick (Priv. comm.) found that in model systems rhizobial populations in sterilized soil and in the rhizosphere of seedlings growing in sterilized soil can be increased when grown in association with other soil isolates. Whether this stimulating effect is operative or not in unsterile soil is not known, but the antagonistic effect on rhizobia by certain soil isolates can be reduced by the presence of the stimulators.

ANTAGONISTIC ASSOCIATIVE EFFECTS

The term antagonism encompasses competition, amensalism, predation, parasitism and lysis whereby one organism inflicts harm to the other, and thereby causes inhibition of growth or restriction of its activities.

Evidence of antagonism excercised by non-rhizobial population of the soil towards rhizobia and their symbiotic performance has been

demonstrated by a number of workers both in the laboratory and in the field. Useful reviews on the earlier works have been made by Fred *et al.*, (1932), Allen and Allen (1958) and Vincent (1954b, 1965).

Competition

Microbial antagonism may be operative through competition, and the ecological significance of competition between soil microorganisms has been discussed by Clark (1965, 1967) and Baker (1968).

Among the limiting factors for which the microorganisms in soil may compete, nutrients appear to be the most important one (Clark, 1965, 1967). Garrett (1965) postulates that "Every soil microbiologist should constantly keep in mind the simple fact that the commonest cause of death in a microorganism in whole or part is straight starvation", in indicating that the supply of food materials in the soil can be said to be always inadequate for microorganisms except in locally rich micro-environments, e.g. the rhizosphere. Keeping this fact in mind, it is not surprising that rhizobia with their complex nutrient requirements will face the competition stress in soil with other saprophytes. Invasion of the legume roots by rhizobia may be an escape from the competition stress that they may confront in the soil.

Rhizobia usually prefer to grow on simple substrates like mono- or disaccharides or sugar alcohols (Allen and Allen, 1958; Graham, 1964) and are unable to utilize high molecular weight compounds like cellulose, lignin or pectin (Fahraeus and Ljunggren, 1967). But the most abundant carbon molecule that enters the soil is cellulose, and the concentration of simple water soluble carbon substrates at a given time in a soil is usually low (Waksman, 1952). Certain rhizobia have been reported to use some aromatic compounds (Hussein, *et al.*, 1974) but phenolic com compounds have been found to be toxic towards rhizobia (Rao and Iswaran, 1973). They can use a number of nitrogen sources including amino acids (Allen and Allen, 1950). In addition, they have specific requirements for vitamins (Graham, 1963a) and unknown growth factors (Allen and Allen, 1950). Micronutrients e.g. molybdenum and cobalt are required by rhizobia for their nitrogenase activity in symbiotic association (see Vincent, 1965).

Competition between rhizobia and soil microorganisms

As early as 1899, Nobbe and Hiltner (see Fred *et al.*, 1932) suggested that the competition between rhizobia and the rest of the microflora of soil may result in harm to rhizobia. Duggar and Purcha (1912) obtained better growth of rhizobia in sterilized than in unsterilized soil suggesting a competition effect. My own experience on the growth of *R. trifolii* and *R. lupini* in sterilized and unsterilized soil also has been the same (Table 1). The general trend of the results in Table 1 indicated a better growth of the rhizobial strains in sterilized soil, particularly when amended with carbon substrates, but no or little growth stimulation

Table 1. The effect of nutrient supplements on the number of *R. trifolii* (WU1) and *R. lupini* (WU 425) in sterilized (7 days incubation) and unsterilized (10 days incubation) Carnamah grey sand at 28°C.

Treatment	Log_{10} number of rhizobia/g soil[1]				
	Sterilized soil		Unsterilized soil		
	R. trifolii	*R. lupini*	*R. trifolii*	*R. lupini*	Final pH
Initial numbers	3.50	3.74	4.59	4.59	-
Unamended	6.21	5.30	4.04	4.04	6.25
Glucose (0.1%)	-	-	4.56	5.12	6.60
Glucose (0.5%)	-	-	4.63	5.63	4.15
Glucose (1.0%)	8.70	5.95	4.12	5.82	4.13
Mannitol	9.07	5.79	-	-	-
Sucrose	8.86	5.84	-	-	-
Sodium glutamate[2]	9.01	5.60	1.00	1.00	7.76
Urea[2]	7.51	5.73	1.00	1.00	8.40
KNO_3[2]	4.12	4.54	4.54	4.30	5.99
NH_4NO_3[2]	5.51	4.61	4.82	4.30	6.04
NH_4Cl[2]	5.67	4.57	4.12	4.82	6.00
Mineral nutrient mixture[3]	6.19	4.59	2.30	3.63	7.35
Trace element mixture[4]	5.89	4.90	4.63	4.63	6.05
Vitamin mixture[5]	6.08	4.88	4.82	4.12	6.05

1. Average of duplicate soil samples, on oven-dry basis. Counts in sterilized soil was done by plate method, and that of unsterilized soil by MPN method (Date and Vincent, 1962).
2. Applied at the rate of 40 mg N per 100g soil.
3. K_2HPO_4, NaCl, $MgSO_4 \cdot 2H_2O$ at the rate of 0.1, 0.1, 0.05 and 0.003g, respectively, per 100g soil.
4. H_3BO_3, $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ at the rate of 20, 16, 6.4 and 3.2 µg, respectively, per 100g soil.
5. Calcium pantothenate, folic acid and thiamine HCl @ 160 µg each and biotin @ 0.4 µg per 100 g soil.

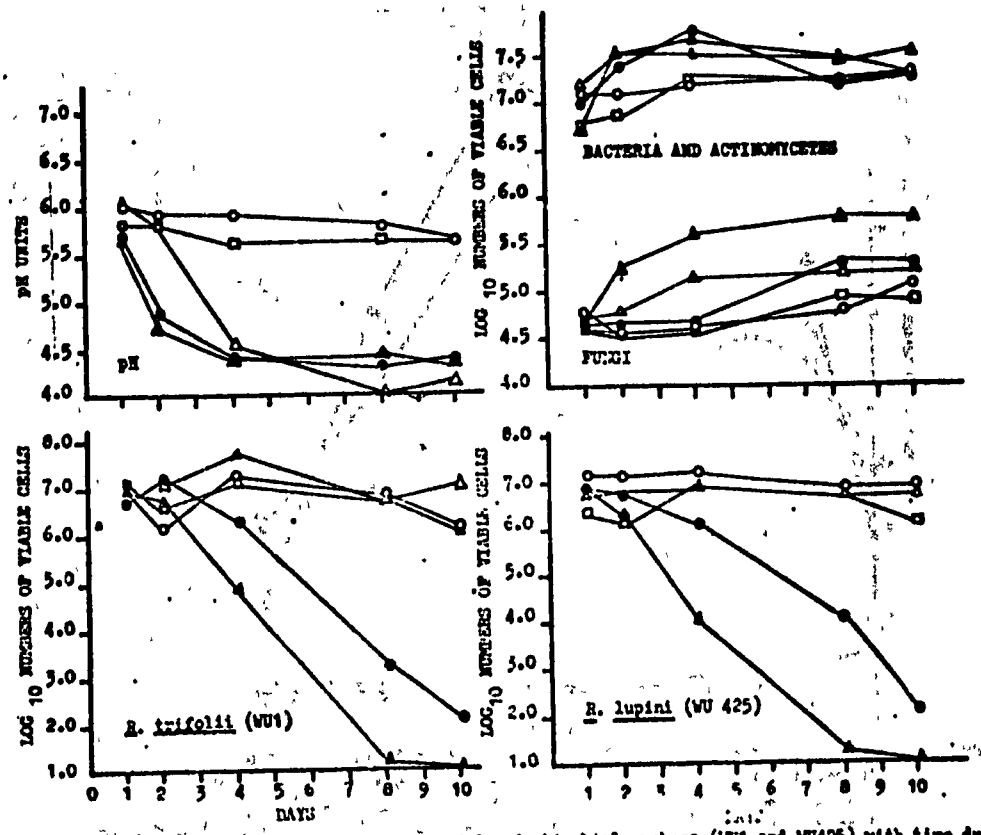

FIGURE 1: Progressive changes in pH, microbial and rhizobial numbers (WU1 and WU425) with time due to amendments in unsterilized Carnamah grey sand (Amendments: o——o control; Δ——Δ glucose; □——□ NH_4Cl; ●——● glucose + NH_4Cl at C/N 60; ▲——▲ glucose + NH_4Cl at C/N 10).

was observed in unsterilized soil. Amendments with glucose, ammonium chloride and both combined at various C/N ratios gave a different set of results. While glucose or ammonium chloride had little stimulatory or no effect on the growth of *R. trifolii* and *R. lupini* in unsterilized soils, a combination of them, particularly at low C/N ratios e.g. 10 and 5, caused a drastic reduction, or even virtual elimination of both species (Chowdhury *et al.*, 1976). Following these observations, the progressive decline of the rhizobial species, the numbers of bacteria, actinomycetes and fungi, changes in pH of the soil, oxygen uptake in soil as well as the presence of antibiotic substance(s) in soil water of the amended soil was followed. The results are summerized in Figs. 1 and 2. No antibiotic substance was detected in the soil for either species of *Rhizobium*. In fact, soil water proved to be stimulatory in certain instances, but the stimulatory action was detectable only between the second and fourth day of inocubation. Soil acidity produced by the amendments could not account for the decline in rhizobial numbers. The addition of calcium carbonate also did not help the rhizobia to persist in the amended soil (Chowdhury, *et al.*, 1976). It was, therefore, deduced that some form of competition between rhizobia and soil microorganisms was operative as will be evident from the marked stimulation of microbial activities in the amended soil during the period of incubation (Fig. 2).

A total of 51 bacteria, 42 yeasts, 50 actinomycetes and 61 fungi were isolated from the combined glucose and ammonium treated soil. Their associative effects (except actinomycetes) on the growth of rhizobia

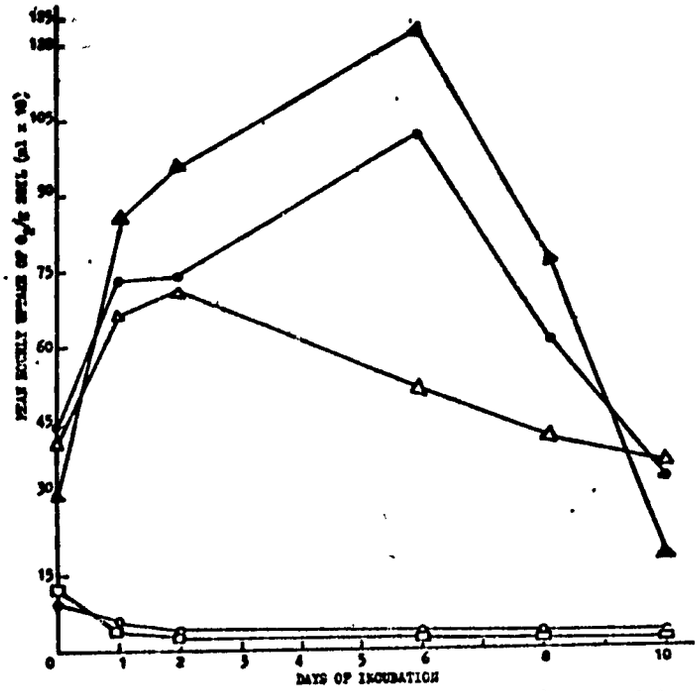

FIGURE 2: Mean rates of oxygen uptake (in µl/hr/g of soil) of amended and unamended soils on different days during the period of incubation (Amendments: o——o control; Δ——Δ glucose; □——□ NH_4Cl; ●——● glucose + NH_4Cl at C/N 60 and ▲——▲ glucose + NH_4Cl at C/N 10).

Table 2. Associative effects of soil isolates on the growth of *R. trifolii* (WU1) and *R. lupini* (WU425) in sterilized unamended Carnamah grey sand incubated for 10 days at 28°C.

Group of soil isolates[1]	Log_{10} numbers of rhizobia/g soil[2]		Final pH
	R. trifolii	*R. lupini*	
Control (without any soil isolate) soil isolate	8.28	8.12	6.30
Mixture of all bacteria and yeasts	6.12	7.30	6.20
Mixture of all fungal isolates	5.63	7.30	6.25
Mixture of all soil isolates	5.82	7.30	6.10

1. Soil isolates refer to 51 bacteria, 42 yeasts and 61 fungi isolated from Carnamah grey sand amended with 1% glucose and 0.15% ammonium chloride.
2. Average MPN counts/g of duplicate soil samples on oven-dry basis, initial log_{10} number of rhizobia introduced are 4.36 and 4.56 for *R. trifolii* (WU1) and *R. lupini* (WU425), respectively.

were examined in sterilized unamended soil (Table 2). The growth of *R. trifolii* strain was substantially reduced by the presence of soil isolates, while that of *R. lupini* strain was somewhat depressed, indicating less competitive ability of *R. trifolii* strain WU1 (= strain TA1). Chatel *et al.*, (1968) also reported the weak competitive ability of the same strain in the same problem soil, they called it weak "saprophytic competence." While the growth curves for various strains of *R. trifolii* and *R. lupini* in sterilized Carnamah grey sand could be obtained within 7 days' incubation (Chowdhury *et al.*, 1968), it was difficult to ascertain the generation times for the growth of *R. trifolii* strain WU1 or *R. lupini* strain WU 425 in the same unsterilized soil within 10 days incubation (Fig. 1).

Dr. M.J. Trinick (priv. comm) from West. Australia made systematic studies on the interaction of 1500 soil isolates with *Rhizobium* species in model systems by introducing one or more soil isolates in sterilized soil along with rhizobia. In model interaction studies rhizobia and some microorganism competed in the soil and rhizosphere and there was a reduction in the population of both organisms. Neither organism showed an inhibitory effect on the other when grown together on synthetic media, nor was any toxin production observed. Sometimes the agar-stimulatory isolates also adversely affected the *R. trifolii* population in the clover rhizosphere without any evidence of toxin production.

Inter-strain Competition among Rhizobia for Nodulation

Competition for growth on the legume root surface by different strains of rhizobia and the different competitive abilities for nodulation between strains of rhizobia have been well documented (Read, 1953; Vincent and Waters, 1953; Johnson *et al.*, 1965; Caldwell, 1969, Marques Pinto *et al.*, 1974; Labandera and Vincent, 1975). Competition between native ineffective strains and effective inoculant strain of clovers sometimes caused serious problem in the establishment and maintenance of improved pastures; such situations have been encountered in Australia (Jenkins, *et al.*, 1954; Vincent 1954a), U.S.A. (Holland, 1970) and Uruguay (Labandera and Vincent, 1975). Competition for nodulation sites between strains of *R. meliloti* (Marques Pinto *et al.*, 1974) and of *R. japonicum* (Means *et al.*, 1961; Caldwell, 1969; Damirgi *et al.*, 1967; Ham *et al.*, 1971) has also been reported. Several serotypes of *R. japonicum* strains are distributed in the soils of U.S.A. (Johnson and Means, 1963; Bezdicek, 1972) and the nodulation of soybean by these serotypes depends upon the selection by the host genotype (Caldwell, 1969; Skrdleta and Karimova, 1969); closely related genotypes being nodulated similarly (Caldwell and Vest, 1968).

Sometimes effective strains of *R. trifolii* were found to be more competitive than the ineffective strains (Robinson 1969a; Marques Pinto *et al.*, 1974) while in other cases, ineffective strains were more successful in nodule formation (Nicol and Thornton, 1941; Vincent and Waters, 1953; Ireland and Vincent, 1968) indicating that competitive ability and effectiveness are quite independant characters of a particular strain. But the host may excercise a strong selective preference for effective strain(s) in a field containing a mixed population of effective

and ineffective strains (Robinson 1969b). Brockwell (1972) observes, if this is correct, "it explains why a nodule on a legume naturalized in the field is almost invariably the most reliable source of bacteria effective for that species." The relative numbers of competing strains of rhizobia in the rhizosphere may not be always important in determining the competitive ability or advantage of a strain. Robinson (1969a) demonstrated that even when the ratio of the ineffective to effective strains was million to one, effective strains still produced nodules, although in smaller numbers. Singer *et al.*, (1964) also observed a similar phenomenon with *R. trifolii*. However, increasing the inoculum size ensured good nodulation by some strains (Johnson *et al.*, 1965; Ireland and Vincent, 1968; Holland 1970). The superiority in numbers of a strain in a mixed inoculant may not be the same representation on root surface of the host. Marques Pinto *et al.*, (1974) expressed the relative success of competing paired strains by "competitive index", defined as the ratio of nodules due to each strain under conditions of equal representation on the root. The results of Labendera and Vincent (1975), however, showed that the competitiveness in nodule formation is not necessarily related to the realtive growth outside the plant.

Caldwell and Weber (1970) observed that the dominance of a particular serogroup in soybean nodules depends on planting date, and this phenomenon was found to be related to root temperature (Weber and Miller, 1972). This relationship can be exploited to give competitive advantage to a particular effective strain by adjusting planting date of the plant.

Table 3. Relative inhibition of *R. trifolii* (WU1) and *R. lupini* (WU425) by 204 soil isolates on agar plates

Isolates	Percent showing relative inhibition[1]							
	Strong		Moderate		Weak		None	
	WU1	WU425	WU1	WU425	WU1	WU425	WU1	WU425
Gram positive[2] bacteria (29)	24.1	17.2	31.0	27.6	24.1	24.1	20.8	31.1
Gram negative bacteria (22)	4.5	18.2	36.4	31.8	31.8	22.7	27.3	27.3
Yeasts (42	14.3	21.4	40.5	14.3	26.2	33.3	19.0	31.0
Actinomycetes (50)	8.0	8.0	18.0	24.0	14.0	18.0	60.0	50.0
Fungi (61)	31.1	39.3	41.0	41.0	11.5	8.2	16.4	11.5

1. Diameter of inhibition zone around soil isolate: strong > 15 mm; moderate, 8-15 mm; weak < 8 mm; none, with no visible inhibition.
2. Number in the parentheses indicates the total number of isolates in that group.

Amensalism

Amensalism is the form of antagonism where one organism produces toxic substances for other(s). Such toxic substances may, in broad sense, be called antibiotics (Park, 1967). As a matter of fact most investigated microbial antagonisms concern the production of inhibiting substances or antibiotics (Park, 1960). This is particularly true in the study of soil antagonists against rhizobia. A number of bacteria, actinomycetes and fungal species from soil and legume rhizosphere have been reported to have an antagonistic effect upon rhizobia as measured by inhibition of rhizobial respiration (Thornton *et al.*, 1949), nodule production, growth and nitrogen content of host plant (Robison, 1945, 1946; Abdel Ghaffar and Allen, 1950; Holland and Parker, 1966) or the growth inhibition or rhizobia in laboratory media or sterilized soil. A greater percentage of the soil isolates from Carnamah grey sand amended with glucose and ammonium chloride at C/N 10 showed strong to weak inhibitory effect on both *R. trifolii* and *R. lupini* in agar medium (Table 3). Among antagonistic bacteria both gram negative and gram positive types are found (Afrikian, 1963; Wieringa, 1963; Hattingh and Louw 1964; 1966a; Kumara Rao *et al.*, 1972; Bhalla and Sen 1973, and Smith and Miller, 1974). The principal antagnostic bacteria are members of *Pseudomonas* and *Bacillus*. Bacteria inhibiting the growth of rhizobia in agar media may not necessarily inhibit nodulation of the host, even at a low inoculum level of rhizobia (Smith and Miller, 1974). Trinick (Priv. Comm) on the other hand observed that agar-inhibiting bacterial isolates could strongly inhibit the growth of rhizobia in soil as well as in host rhizosphere without themselves being affected.

The antibiotic effect of actinomycetes upon rhizobia has been demonstrated by many investigators (Wieringa, 1963; van Schreven, 1964; Holland and Parker, 1966; Damirgi and Johnson, 1966; Patel, 1974). These antagonists are members of the genus *Streptomyces*, and their anti-rhizobial effects may be strain-specific (Patel, 1974). The antagonistic actinomycetes can reduce nodulation of the host (van Schreven, 1964; Damirgi and Johnson, 1966).

A number of fungal species are reported to inhibit rhizobia. Among them, species of *Penicillium* and *Aspergillus* are found to be most active (Robison, 1945; Thornton *et al.*, 1949; Vyas and Prasad, 1960; Holland and Parker, 1966; Gupta 1973). Species of *Alternaria, Cephalosporium, Fusarium, Paecilomyces, Thielvia* and many others have also been found inhibitory to rhizobia (Subba Rao and Vasantha, 1965; Chonkar and Subba Rao, 1966; Sethi and Subba Rao, 1972; Gupta, 1973). The inhibitory action on *R. trifolii* and *R. lupini* in agar medium of 61 fungi isolated from Carnamah grey sand of West. Australia is presented in Table 4. Some of the isolates were found to be specific in their antagonistic action towards *R. trifolii* or *R. lupini*. Colonization of soil or rhizosphere of the legumes by antagonistic fungi resulted in poor symbiosis in terms of nodulation and plant growth (Robison, 1945; Holland, 1962; Holland and Parker, 1966), leghaemoglobin in the nodules and plant nitrogen content (Chonkar and Subba Rao 1966; Sethi and Subba Rao, 1972).

Table 4. Number of fungal isolates inhibitory towards *R. trifolii* (WU1) and *R. lupini* (WU425) on agar plates.

Fungi	Degree of inhibition[1]							
	Strong		Moderate		Weak		None	
	WU1	WU425	WU1	WU425	WU1	WU425	WU1	WU425
Penicillium spp. (27)[2]	13	26	6	6	1	1	7	4
Aspergillus spp. (15)	4	4	6	8	2	1	1	1
Fusarium spp. (7)	0	2	7	5	0	0	0	0
Mucor spp. (5)	2	2	3	3	0	0	0	0
Pullularia spp. (3)	0	0	2	1	0	1	1	1
Cladsporium spp. (1)	0	0	0	1	0	0	1	0
Curvularia spp. (1)	0	0	1	1	0	0	0	0
Stemphylium spp. (1)	0	0	0	0	1	0	0	1
Unidentified (1)	0	0	0	0	1	1	0	0

1. Diameter of inhibition zone around the growth of soil isolate: strong> 15mm; moderate, 8-15mm; weak < 8mm; none with no visible inhibition.
2. Number in the parentheses indicates the total number of isolates in that genus.

The inhibitory effects and antibiotic production against rhizobia by soil antagonists are often dependant on nutritional status of the soil. Amendment with suitable carbon sources stimulates the inhibition of rhizobia and their symbiotic performance (Holland and Parker, 1966; Hattingh and Louw, 1969b). The nature of nutrient amendment of soil may also affect the nature of antibiotic(s) produced. The inhibitory effects of soil isolates upon the survival of *Rhizobium trifolii* *Rizobium lupini* in sterilized amended soil are shown in Tables 5 and 6. In glucose amended soil, some rhizobial cells could survive despite antibiotic production (Table 5); probably the antibiotic activity was bacteriostatic in nature. But the rhizobial population was almost eliminated from soils treated with glucose and ammonium chloride (Table 6), indicating the production of strong bactericidal antibiotics. Stimulation of antibiotic production in soil due to nutrient amendments has been observed by many workers (See Gray and Williams, 1971). Hockenhull (1963) considered the biosynthesis of antibiotics as the result of a "series of inborn errors of metabolism" of the causative organism; such errors could be aggravated by the over supply of nutrients. If nutrient abundance favours antibiotics production, the possible sites of their production in natural soil would be the readily decomposable organic debris and, more probably, the rhizosphere where soluble organic substances like sugars, amino acids and certain growth factors may occur as root exudates (Rovira 1962). The occurrence of antibiotic producing organisms against rhizobia in the host rhizosphere or on their root surface (Hattingh and Louw 1964, 1966a, 1969a; Mallik and Hussain, 1972; Bhalla and Sen, 1973; Smith and Miller, 1974) as well as on nodule surface (Subba Rao and Vasantha, 1965) has been well documented.

Table 5. Associative effects of soil isolates on the growth of *Rhizobium trifolii* (WU1) and *R. lupini* (WU425) in sterilized Carnamah grey sand amended with 1% glucose alone and incubated for 10 days at 28° C.

Soil isolate groups[1]	Log_{10} numbers of rhizobia/g soil[2]		Final pH	Inhibition of rhizobia by soil water[3]	
	R. trifolii	*R. lupini*		*R. trifolii*	*R. lupini*
Control (without any soil isolate)	9.30	8.63	6.13	0	0
Mixture of all bacteria and yeasts	3.63	3.30	4.26	0	0
Mixture of all fungal isolates	3.56	4.30	3.89	+	+
Mixture of all soil isolates	4.12	4.30	3.98	+	+
Table 6. Same as above, but amended with 1% glucose and 0.15% ammonium chloride.					
Control (without any soil isolate)	7.82	7.30	6.00	0	0
Mixture of all bacteria and yeasts	1.00	1.00	4.13	+	0
Mixture of all fungal isolates	1.00	1.00	3.93	+	+
Mixture of all soil isolates	1.00	1.00	3.80	+	+

[1] Soil isolates refer to 51 bacteria, 42 yeasts and 61 fungi isolated from Carnamah grey sand amended with 1% glucose and 0.15% NH_4Cl.

[2] Average MPN counts/g of duplicate soil samples on oven dry basis; initial log_{10} numbers introduced are 4.77 and 5.08 for *R. trifolii* and *R. lupini* respectively.

[3] 0 = no inhibition; + = inhibition. Soil water was extracted by centrifugation of wet soil samples at 1000g through a membrane filter in a Hemming filter assembly.

The ecological significance of antibiotics inchanging the composition of soil microflora in nature has been a matter of controversy (see Alexander, 1961; Gray and Williams, 1971). Yet, there is evidence that active anti-rhizobial factors are formed in nature, although their exact chemical nature, relative persistence and activity in the soil are not definitely known. Casas-Campillo (1947) obtained either- and alcohol- extracts froj soils cropped to legumes exhibiting antibiotic activity against four species of *Rhizobium*. Chatel and Parker (1972) reported the occurrence of a toxic factor, transient in nature and active against *R. trifolii* in the water from a Western Australian soil, where second year noduiation problem with clover but not with lupin was evident. The same extract did not inhibit *R. lupini*. The anti-rhizobial factor may be of microbial origin (Chatel and Parker, 1972) or of plant origin (Parle, 1964; Rice, 1964). Root exhudates (Rice, 1968) and leaf leachates (Blum and Rice 1969; Rice, 1971) from the pioneer plant species e.g. *Euphorbia supina* in some abandoned fields of several mid Western States of the U.S.A. were found to inhibit

rhizobial growth and host nodulation. The inhibitory substance(s) was identified to be tannic acids (Blum and Rice, 1969) or a mixture of gallotannins (Rice, 1965). Virtanen and Linkola (1948) considered that the volatile fraction of the antibiotic produced by *Bacillus mesentericans* to be inhibitory to *R. leguminosarum*, *R. phaseoli* and *R. trifolii*, whereas Mallik and Hussain (1972) reported the anti-rhizobial factor from various antagonists to be thermostable and not adsorbed by activated charcoal.

The strains from different species of *Rhizobium* have different sensitivity towards pure antibiotics; slow-growing rhizobia being less sensitive than the fast-growing rhizobia to certain antibiotics at low concentrations (Graham, 1963b). The antibiotics inhibiting rhizobial growth also inhibit the growth of the host but the degree of inhibition depends on the type and concentration of the antibiotic (Melkumova, 1962, Visona and Pesce, 1963). Recently Levin and Montgomery (1974) found that there was no dramatic difference either in infectivity or in effectiveness between certain antibiotic-sensitive strain and their resistant mutants of *R. japonicum*, but other workers report loss of effectiveness in some antibiotic-resistant mutants (Schwinghamer, 1964, 1967; Zelazna-Kowalska, 1971).

Inter-species or even inter-strain amensalism among rhizobia may also be operative. Schwinghamer and Belkengren (1968) isolated a relatively heatstable polypeptide type of antibiotic from an ineffective *R. trifolii* strain (T24). This antibiotic was found to be active against a number of strains from different species of *Rhizobium*. They further demonstrated when T24 is present in a mixed inoculum with effective strains of *R. trifolii*, the nodulation of red clover by the effective strains was greatly reduced. Although the antibiotic was active on several strains of *R. leguminosarum* in agar culture, the antibiotic producer had no effect on the nodulation of pea by effective *R. leguminosarum* strain.

Roslycky (1967) reported that certain rhizobial strains are capable of producing bacteriocins which were later identified to be phage-like particles but incapable of reproducing (Lotz and Mayer, 1972; Schwinghamer, *et al.*, 1973). Such potent bacteriocinogenic strains are common in natural soil (Schwinghamer, 1971), and among them strains producing short-type bacteriocin (Type IIa and IIB) are probably more important under natural situations. As many as 24 to 65 percent rhizobial strains from different localities of south-eastern Australia are reported to be antagonistic to other strains through mild antibiosis or bacteriocinogeny (Bergersen *et al.*, 1971).

EXPLOITATION

(Predation, Parasitism and Phage Action)

Park (1960) coined the term "exploitation" to include the antagonistic effects of predators, parasites and viruses. These antagonists are known to take part in maintaining biological equilibrium in soil.

As well they may participate in the biological control of plant pathogens in soil (Boosalis and Mankau, 1965; Baker, 1968). Their influence on rhizobial population in soil and on symbiosis has been little studied.

Bdellovibrio strains predatory to *Rhizobium* have been isolated by Parker and Grove (1970) and by Keya and Alexander (1975a). The predatory action of a number of protozoa (e.g. *Amoeba, Hartmanella, Naeglaria, Tetramitus)* upon rhizobia has been demonstrated by Danso *et al.*, (1975) in model systems but in all cases predation was host-density dependant. The ecological significance of *Bdellovibrio* and protozoa is still obscure. However, the results of Keya and Alexander (1975a, b) and Danso *et al.*, (1975) indicate that the predation by such antagonists may not be alarming under natural conditions. To the best knowledge of the author, there is no reported evidence of predation of rhizobia by either myxobacteria or myxomycestes.

Rhizobiophages for different species of *Rhizobium* have been detected in soils of diverse properties and cropping history. Kowalski *et al.*, (1974) reported as many as 10^3 phage particles for *R. japonicum* in soil. In the senescent nodules the number was as high as 3×10^5; active nodules had only few particles. Despite widespread occurrence of rhizobiophages, their ecological significance in "militating against the survival and functioning of rhizobia in the soil has yet to be unequivocally demonstrated" (Vincent, 1965). Lysogeny in rhizobial strains has been observed by Marshall (1956), Takahashi and Quadling (1961), Schwinghamer and Reinhardt (1963). The lytic action of temperate phate from lysogenic strains upon other strains of rhizobia might be important in the survival of rhizobia in multi-strain inoculants, and perhaps, in the soil.

POST INFECTION ANTAGONISM

Despite successful nodulation, the full benefit of symbiosis may not be realized due to certain biotic influences. They include the parasitic attack by insects, nematodes and viruses upon the host and/or nodule. The larvae from the eggs of many soil insects may feed upon the nodules or root hairs of the host. In this respect bean and pea weevil (*Sitona lineatus)*, large lucerne weevil *(Otiorrhyschus ligustici)* (see Mishustin and Shil'nikova, 1971) and clover root weevil *(Amnemus quadrituberculatus)* (Mears, 1967) are noteworthy. Diatloff (1965) observed that the larvae of *Rivellia* (Diptera) could damage 60-70% functional nodules of *Glycine javanica*, and severely interfered with nitrogen fixation.

Many workers have found a number of parasitic nematodes in the root zone of various species of leguminous plants (see Mishustin and Shil'nikova, 1971). Some of them e.g. species of *Heterodera* and *Meloidogyne* are reported to reduce nodulation and root hair production in soybeans (Lehman *et al.*, 1971; Balasubramanian, 1971) and some (e.g. species of *Ratyllenchus)* interfere with the functions of the roots and nodules of bean, lucerne and clover (see Mishustin and Shil'nikova, 1971). On the other hand, Taha and Raski (1969) found

that nematodes did not interfere with nodule formation or with the nitrogen fixation efficiency. I have observed the root-knot nematode *(Meloidogyne* spp.) in soybeans at Morogoro, Tanzania and the infested plants had comparatively fewer nodules than uninfested plants.

Virus infections of leguminous plants are reported to interfere with nodulation and nitrogen fixation. For example, infection of white clover by clover phyllody virus (CPV) resulted in the production of ineffective nodules when inoculated with normally effective *R. trifolii*. Some plant viruses seemed to exert more harmful effect on the symbiotic system (nodule number, size and nitrogen fixation) than on the plants (Blaszczak *et al.*, 1974). On the other hand, Rajagopalan and Raju (1972) reported *Dolichos* enation mosaic virus to enhance leghaemoglobin formation and nitrogen fixation during early growth of *Dolichos lablab* in sand culture.

FUTURE RESEARCH

It is apparent from the foregoing discussion that the biotic influences upon symbiosis may be operative from the pre-infection to post-infection phases.

During the pre-infection phase, non-rhizobial soil antagonists may result in the partial to complete failure of symbiosis by affecting the rhizobial survival and proliferation in the host rhizosphere through (a) competition for nutrients, (b) production of toxin or antibiotic active against rhizobia and (c) predation and parasitism on rhizobia. Most of the studies concerning these forms of antagonism have been made under laboratory conditions by isolating the soil organisms and studying their effect upon rhizobia in agar media or in model systems using sterilized soil. Studies in model systems involved the enumeration of rhizobia by using suitable marker-strains e.g. antibiotic-resistant strains (Danso and Alexander, 1974) or by plant-infection technique (Wilson, 1926, Date and Vincent, 1962; Brockwell, 1963b). Antibiotic-resistant strains can be enumerated easily in artificial medium and may be useful in competition studies but their usefulness in amensalism studies in doubtful. Plant-infection method is laborious and time-consuming. The recent immunofluorescent technique by Schmidt (1974) for autoecological studies appears promising, but more work will be needed to confirm the reliability of the method.

The evidence of decline in rhizobial numbers in the preinfection phase due to competition for nutrients by soil non-rhizobial organisms is rather indirect. The limiting factor for which competition may occur, as demanded by Milne (1961), has not been yet defined. Danso and Alexander (1974) failed to attribute competition as a causative factor for consistant decline of rhizobial numbers in unsterilized soil, unamended or amended with organic substrates. Such an approach to search for "limiting nutrient" should be made with care. Addition of a nutrient to stimulate rhizobial numbers in the rhizosphere (Bergersen *et al.*, 1958) may also stimulate a section of the microflora in the complex biocommunities of soil to result in severe competition

Figs. 1 and 2) or stimulate antibiotic production (Chowdhury *et al.*, 1976).

The occurrence of organisms producing antibiotics towards rhizobia is well documented, yet such organisms have been found to occur in soils having no apparent nodulation problem (Patel, 1974). Further, the antagonistic or stimulatory action of soil isolates on *Rhizobium* in agar medium is often dependant on the composition of medium (Smith and Miller, 1974) or on the quality of agar (Hattingh and Louw, 1965). Thus the ecological significance of such organisms demands careful interpretation. There are, of course, proofs that anti-rhizobial factors of microbial origin may occur in detectable concentrations in natural soil. Even if such substances may be transient in nature (Virtanen and Linkola, 1948; Chatel and Parker, 1972), they might cause sufficient damage to rhizobial population to result in nodulation problems. But the chemical nature of these toxins or antibiotics is yet to be determined for better understanding of their effect on rhizobial ecology.

The control of such soil antagonists by soil sterilization or fumigation (Cass-smith and Holland, 1958; Khan *et al.*, 1968) is a possible remedy, but aging of soil (Cass-Smith and Holland, 1958) appears to be practical agricultural husbandry, especially for farmers of developing countries. The use of antibiotic-resistant strains possessing symbiotic effectiveness (Levin and Montgomery, 1974) deserves consideration. *Rhizobium* strains with antibiotic or bacteriocinogenic property (Schwinghamer and Belkengren, 1968; Schwinghamer 1971) appears to be another interesting field of research for their useful exploitation against microbial antagonism in the inoculant as well as in the field (Bergersen *et al.*, 1971). The use of adhesives, e.g. gum arabic (Subba Rao *et al.*, 1971), energy materials e.g. sucrose (Parker, 1962) or palleting legume seeds (Brockwell, 1963a) need to be exploited more as a measure of protection of the inoculants against soil antagonists. The microorganisms with stimulatory effects towards rhizobia could also be employed to promote effective symbiosis. Unfortunately, this aspect has escaped our attention or being ignored despite the suggestion by Vincent (1954b) to explore the possibilities.

During the infection phase, antagonism in the form of competition for nodulation sites on the host root surface by the effective and ineffective strains may lead to serious consequences. Methods (Brockwell and Dudman, 1968) and rates (Ireland and Vincent, 1968) of inoculation may improve upon the situation, but the mechanism of such competition is still little understood (Labandera and Vincent, 1975).

Plant pathogens including viruses, insects and nematodes are potential antagonists on symbiosis during post-infection phases. The effect of plant pathogens upon symbiosis has not received much attention. Orellana *et al.*, (1976) demonstrated that *Rhizoctonia solani*, causing root-rot in soybean, diminished nodulation and nitrogen fixation in inoculated soybean. The application of chemicals as protective measure to control the insects and nematodes demaging the legume roots and nodules (See Mishustin and Shil'nikova, 1971) needs careful consideration because of the possible harmful effects of such plant protection

Table 7. Persistence in podzolic soils in South-eastern Australia of two strains of *R. trifolii* in approximately equal numbers by clover seed inoculation during pasture establishment (After Brockwell *et al.*, 1968).

Age of pasture	No. of isolates examined	Proportion of isolates recognized		Unknown
		TA1	UNZ29	
1 year	130	68	12	20
1-2 years	226	44	1	55
2 years	20	25	0	75

chemicals upon rhizobia (see Smith, 1973 for extensive references). The report by Joshi and Carr (1967) that clover phyllody virus may induce the effective rhizobia to be transformed to ineffective forms which may even spread in the soil poses a potential danger. Similarly, mutation of effective strains into ineffective forms due to repeated exposure to antibiotic producing organisms (Van Schreven, 1964) may also occur in a natural soil where rhizobia are living with other microorganisms including the antibiotic producers. Whether such transformations are widespread in nature is not known.

The differences among rhizobial strains in their "saprophytic competence" has been demonstrated by Chatel *et al.*, (1968) but the mechanism for better saprophytic competence for a rhizobial strain is not well understood. Brockwell *et al.*,(1968) reported that there is a tendency for the inoculatn strain to disappear from the soil *Rhizobium* population, if not immediately after inoculation but definitely in the course of time (Table 7), supporting the importance of saprophytic competence of the inoculatn strains. Further research on this criterion for strain selection in order to establish the introduced rhizobia in the soil in sufficient numbers for effective nodulation of the legumes is warranted. Inoculation of the preceeding non-legume crops with rhizobia (Diatloff, 1969) may be another possibility for successful soil colonization by inoculant strain. In the study of ecology of rhizobia, the effects of environmental and soil conditions on soil population as well as on rhizobia should be considered together because environmental and edaphic conditions also regulate the biotic populations including the stimulators and antagonists for rhizobia.

The evidence on the adverse effects of soil antagonists on symbiosis is rather indirectly based on the survival and persistence of rhizobia in the soil or rhizosphere, or on the nodulation of the hosts. In certain instances, nitrogen contents of hosts have been determined, but attempts embracing counting rhizobial numbers, nodulation performance as well as nitrogen-fixing capacity have not been made due to practical difficulties. How rapid nitrogenase assay by acetylene reduction technique is available (Dart *et al.*, 1972). Emuneration and

identification of rhizobial strains in the field by suitable technique e.g. immunofluoresence with concomitant assay for nitrogenase activity could possibly give a better idea on the ecology of rhizobia and their symbiosis with the host.

CONCLUSIONS

The members of the genus *Rhizobium* do not have any resting phase and they must persist as saprophytes in the rhizosphere and in the soil to effect successful symbiosis. In the complex soil biocommunities, they confront both friends and foes in addition to environmental stress, which in turn might influence not only the composition of the community but also their behaviour. Antagonists in the form of competitors for nutrients or among rhizobia for nodulation sites, toxin producers, predators and parasites as well as those which can modify the symbiotic effectiveness for nitrogen fixation are present in the soil. The effects of such antagonists on symbiosis *per se* is little studied, but their effects on *Rhizobium* has been recorded by some workers. Despite the presence of antagonists, perhaps in every soil, *Rhizobium* strains do survive,enter into successful symbiosis with their hosts and even persist in sufficient numbers in the absence of their host in most soils. Yet the adverse effects of antagonists are not uncommon. It is, therefore, necessary not only to understand the legume-*Rhizobium* interaction, but also the interactions involving legume-*Rhizobium* - soil conditions including the biotic influences for proper exploitation of the legume-*Rhizobium* symbiosis. Our knowledge in the field of latter interactions is limited, and, perhaps, meagre by comparison to that in other aspects of the symbiosis. But employing the advanced tools and methods, the ecology of *Rhizobium* in the soil should be better understood through further research and they can be exploited to the fullest benefit in practical agriculture.

ACKNOWLEDGEMENTS

The author is greatful to the NifTAL Project, University of Hawaii for kindly providing him with travelling and other facilities, to the University of Dar es Salaam for permitting him to attend the workshop, and to Professor C.A. Parker of the University of Western Australia for kindly reviewing this paper.

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INOCULANTS FOR INDIA

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INTRODUCTION

The earliest documented evidence of the production of inoculants from isolated *Rhizobium* strains in India is that of Madhok (1934). Soil, harbouring viable rhizobia, is a natural inoculant and there are reports that it was being supplied in small packets to farmers for inoculating lucerne *(Medicago sativa)* and berseem *(Trifolium alexandrinum)* in the early 1930's (Motiramani, D.P., pers. comm.). Systematic attempts to build up a country-wide legume inoculant potential are, however, recent and may be traced back to about 15 years. In comparison the usage of inoculants has been an established practice for about 75 years in the U.S.A. (Date, 1974, Burton, 1967).

RELATIVE IMPORTANCE OF PULSES AND OTHER LEGUMES IN INDIA

Legume inoculant technologies in U.S.A., Australia and Europe have achieved a good degree of sophistication over the years through modern techniques and intensive research. India, on the other hand, has yet to achieve this sophistication, and may be considered to be still undergoing development. A broad scientific base has, however, been laid over the past several years with regard to the basic work on strain isolation, strain screening and characterization, crop yield studies, All India Co-ordinated studies, serological investigations, carrier material studies for peat substitution, culture media studies for scaled up productions and investigations on the keeping qualities of inoculants, etc. Adoption of the U.S., Australian and European technologies has not been immediately possible in view of the numerous difficulties posed by different cropping patterns, agronomic practices and agroclimatic conditions. It became inevitable, therefore, that work had to be undertaken to develop inoculation technologies suited to local conditions.

Whereas pasture legumes played a predominant role in the agricultural economy of Australia and soybeans in that of the U.S.A., in India the predominant role is played by the pulses. These include a group of grain legumes of which the most important are *Cicer arietinum* L. (chick-pea or gram), *Cajanus cajan* (L) Millsp. (pigeon-pea or red-gram), *Phaseolus mungo* (L.) (black-gram or 'urad'), *Phaseolus aureus* L.

(green gram or mung), *Lens esculenta* (lentil or 'masoor'), *Pisum sativum* L. (peas or 'matar'), *Vigna sinensis* (Cowpea or lobia) and some other pulses. The area under different pulses is approximately 23 million hectares (Table 1). These form an essential feature of the daily Indian diet, being eaten as "dhal" and constitute the chief source of protein nutrition. Groundnut is cultivated on another 7 million hectares approximately and soybeans on about 100,000 hectares. These, together with the forage legumes, cover approximately 33 million hectares of arable land in the country. Chickpea and pigeon pea are the most important rabi (winter season) and kharif (rainy season) pulses respectively. Mung and urad are believed to be Indian in origin (Singh and Virmani, 1974) and are also very important. Since they did not hold much importance in the better developed countries, little research information was available on these crops. In India they have become 'traditional' crops and the soils harbour large populations of efficient as well as inefficient rhizobial strains which make it difficult for the inoculated strains to establish, thus giving little or even negative yield responses to inoculation in certain areas (Gaur *et al.*, 1974).

Table 1. Area, production and productivity of important legume crops.*

PULSES, with % of total area		Area in 1000 Ha. 1964-65	1970-71	Production in 1000 tonnes	Productivity Kg/Ha.
KHARIF Crop (rainy season)					
Arhar	11.4	2500	2647	1841	695
Moong	9.3	1940	2087	592	284
Moth	8.0	1680	1779	469	264
Urd	8.7	2090	1963	534	272
Kulthi	7.2	1620	1622	487	300
RABI Crop (winter season)					
Gram	35	8900	7810	5247	672
Peas	40	1120	912	913	1000
Lentil	3.5	840	789	376	476
Khesari	8.4	1993	1856	870	469
Misc.	4.5	1110	958	246	256
Groundnut		7167	7326	5000	690
Soybean		-	96	90	900
Forage legumes.					
Guar					
Lucerne					
Berseem		Not recorded separately			
San hemp					

*Ministry of agriculture, unpublished data.

EARLIER WORK AND ESTABLISHMENT OF NEED FOR INOCULATION

Most of the information on Indian rhizobia has come from the different research centres in the country. Subba Rao and Sen (1976) have recently reviewed the work done in India on rhizobiology. The earlier works on nitrogen fixation by Madhok (1940), Sen and Vishwanath (1943), Acharya *et al.*, (1953), Sen 1966), Rangaswamy and Oblisami (1962), followed by the reports of Sundara Rao (1971, 1973, 1974), Sundara Rao *et al.*, (1974), and Bhatnagar (1971) have served to focus attention on the importance of using legume symbiotic nitrogen fixation for increasing crop yields in India. Yield increases due to inoculation were reported in different legumes by Sen and Sundara Rao (1954); pigeon pea (Rangaswami and Nair, 1965); chickpea (Sen, 1966; Bhargava *et al.*, 1974; Kumar *et al.*, 1976); soybean (Balasundaram, 1974; Singh and Saxena, 1972); pulse crops (Rewari *et al.*, 1972); soybean and pulses (Lahiri, 1974; Saxena and Tilak, 1974); groundnut (Singh *et al.*, 1974); and soybeans (Anonymous, 1973). Recently Dube (1976) and Subba Rao (1976) have reviewed yield responses of different legume crops through inoculation. Whereas with soybeans effective inoculation gave spectacular increases in yields due to the absence of *Rhizobium japonicum* from soils of Indian plains, yield responses with traditionally grown crops, though positive in most cases, were observed to be negative in some areas. Localities where chickpea showed no response to rhizobial inoculation, fertilizer nitrogen doses also showed no response; indicating thereby that nitrogen was not the limiting factor in these soils (Sundara Rao, 1973). Inoculation of groundnut gave negative and even deleterious yield responses (Gaur *et al.*, 1974) which has been interpreted as due to the high degree of symbiotic promiscuity in groundnut as well as its rhizobium symbiont, allowing thereby heavy competition from native rhizobia in soil. Dube J.N. (pers. comm.) has observed 30% yield increase in a plot experiment with groundnut. Some of these reports also incorporate the survey work on the performance of native and isolated rhizobia at different localities with new (soybean) as well as traditional (pulse) crops. As pointed out by Date (1976) the establishment of the need to inoculate must precede any inoculant development programme.

CARRIER MATERIALS AND THEIR STERILIZATION

An immediate necessity in the production on inoculants is the availability of a suitable carrier. Iswaran *et al.*, (1972) tried Indian peat obtained from Ootacamund and found it to be suitable for *Rhizobium* survival. Lund *et al.*, (1973) also reported the survival of high population of rhizobia for three weeks or more in such peats. However, Indian peat has less organic matter and is of poorer quality in comparison to the American and Australian sedge peats

(Thomas *et al.*, 1974). Although peat still remains unchallanged as a carrier (Strijdom and Deschodt, 1976), the poor quality and limited availability of Indian peat necessitated a search for a suitable alternative carrier and lignite was suggested (Kandaswamy and Prasad, 1971). This was tried out for soybean inoculants (Dube, 1972, 1973) and found suitable. Sharma and Verma (1973) reported that lignite neutralized with calcium carbonate and supplemented with 10% lucerne meal worked as well as American peat. Sharma and Tilak (1974) investigated the comparative efficiency of lignite, sand, farm-yard manure, soil, and peat with respect to soybean inoculants and observed promising results with lignite. Iswaran *et al.*, (1972) reported finely ground sterilized fallen leaf compost supplemented with minerals to be satisfactory for supporting rhizobial population. Khatri *et al.*, (1973) reported rice husk and fine sand in equal volume to be also satisfactory for several rhizobial species. However, other workers (Sharma and Tilak, 1974) observed that sand and soil based inoculants performed poorly. In India, sand, soil, agar, peat, farm-yard manure (FYM), and lignite based carriers are presently in use (Subba Rao, 1972, 1973). Even penicillin waste, spent ground-coffee waste and cellulose have been considered as carriers. Despite the fact that coal based carriers have recently been reported to perform as well or even better than lignite carrier (Dube *et al.*, 1975) the majority of the Indian inoculant manufacturers are using lignite as carrier for commercial production. Lignite as obtained from the mines is acidic in nature and has to be neutralized with calcium carbonate. Different levels of neutralization may be obtained with and without steam sterilization (Fig.1).

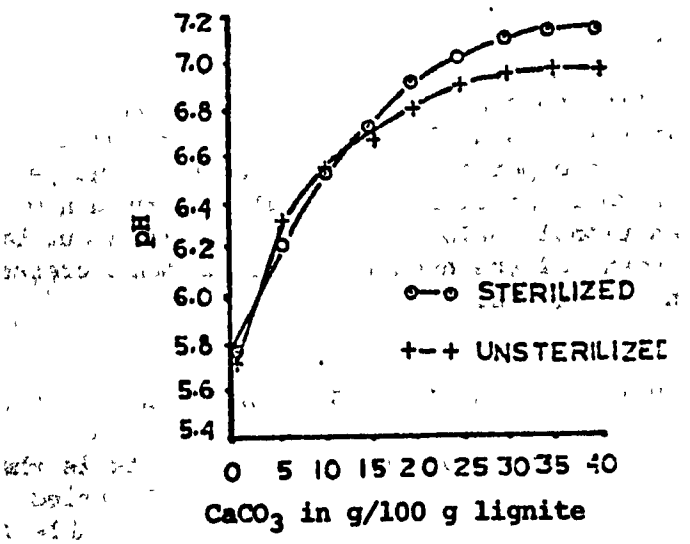

Figure 1. pH values of lignite available from Neyveli mines before and after amendment with varying quantities of $CaCO_3$ under sterilized and unsterilized conditions.

In U.S.A. peat is 'flash' heated in rotating drums with dry air jets at 650°C (Burton, 1967) while in Australia the carrier is sterilized by gamma irradiation (Date, 1974). Whereas the American method provides partial sterilization, the Australian method has aimed at complete sterilization. The latter is, however, not possible with either gas or gamma ray sterilization (Date, 1974). Since carrier sterilization by these methods does not seem to be feasible or practical under Indian conditions, most of the manufacturers are resorting to steam. Gamma irradiation service is an expensive and difficult matter in India and the variations in the moisture content of lignite, due to storage and transport over long distances, makes flash drying dangerous and difficult due to the explosive nature of lignite. The difficulty with steam sterilization is that it requires an autoclavable polythene film (Date, 1974) and in the opinion of this author, may not be the ultimate answer. Contaminants in lignite carrier have been observed to develop even after sterilization at 40 lb. for two hours in punctured tin containers (Bactogin Laboratories, unpublished data). This would make it difficult for manufacturers to observe the Indian Standards Institution (ISI) standards (presently under draft) of 10 per cent contamination as the maximum allowable limit. Some of the inoculants produced on unsterilized or partially sterilized peat are of high quality (Burton, 1967; Vincent, 1974) and whether any advantage is to be gained by complete sterilization needs further examination. Some bacteria are harmlessly associated with nodules and rhizobia (Hedge and Bagyaraj, 1974), and might sometimes appear as contamination. It is believed that contaminants which are not antagonistic to the survival and effectivity of rhizobia do not affect the quality of inoculants (Burton, 1967). Very little literature is available on the relative efficiency of the various sticking materials and their effect on Indian rhizobia. Stickers not only serve to make the inoculant material stick to the seed coat; they also provide protection to rhizobia on the seed coat (Brockwell, 1962). The majority of commercial manufacturers incorporate neutralized gum arabic while others use carboxy-methylcellulose as recommended by Vincent (1970), singly or in mixed form at 5 per cent level. A good sticker increases the inoculant load on the seed surface and ensures effective nodulation.

MODES OF INOCULATION, PELLETING AND INOCULANT LOAD

Burton (1976) had discussed the different modes of seed inoculation and their effects on Rhizobium survival. In India different modes of inoculation have been tried to improve nodulation. *Rhizobium* sprays at the time of flowering have been attempted by Gupta *et al.*, (1973); Jain and Rewari (1974) and although reported to have given yields comparable to seed-inoculated crops, this method has not found practical use. Balasundaram (1974) recorded that attempts at inoculation of wheat with *Rhizobium japonicum* were of little practical value in nodulating soybeans in the successive season. Pelleting with lime has been examined and found to improve nodulation in different crops

(Dadarwal and Sen, 1973; Sethi and Subba Rao, 1969; Chhonkar and Subba Rao, 1966; Subba Rao and Vasantha, 1965; Saxena *et al.*, 1975). Iswaran and Jauhari (1970) reported calcium glycerophosphate to be best for pelleting soybeans. Iswaran and Chhonkar (1971) recommended pelleting of pea with basic slag or lime in acid soils and of green-gram with calcium carbonate in alkaline soils. Presoaking of pea and bengal-gram seeds removes seed-coat toxins and has given better nodulation, growth and nitrogen fixation (Sen *et al.*, 1972). Soybean seeds pelleted with sodium glutamate were observed by Iswaran *et al.*, (1971) to increase nodulation. However, no manufacturer is at present advocating pelleting, and direct seed inoculation still remains the common practice. The key to the successful performance of inoculants lies in the numbers of rhizobia that stick to the seed coat and their subsequent survival in the rhizosphere. Seed coats of leguminous seeds vary greatly in structure and composition (Burton, 1976) and would require different inoculant loads for effective inoculation. Very little literature is available on this aspect for the various grain legumes in India. The American inoculant standards stress a minimum requirement of 300 rhizobia per seed while the Australian standards require 100-1000 rhizobia per seed (Date, 1974). In practice, however, the actual

Table 2. Showing counts of different rhizobia in 2 and 6 month old inoculants and recovery of viable cells from seed treated with expired inoculants.*

Type	Two month old inoculant No./g seed	Six month old expired inoculant No./g seed	Recovery from seed treated with expired inoc. (No./g seed)
Cicer arietinum (Kabuli)	12×10^9	185×10^7	17500
Cicer arietinum (Bengal gram)	12×10^9	185×10^7	10000
Arachis hypogaea (groundnut)	7×10^9	80×10^7	12500
Lens esculenta (lentil)	8×10^9	12×10^7	7500
Pisum sativum (peas)	9×10^9	105×10^7	10000
Trifolium alexandrinum (berseem)	5×10^9	50×10^7	1900
Glycine max (soybean)	9×10^9	165×10^7	20000

* Bactogin Laboratories (unpublished data).

requirements are often much more (Burton, 1976). Higher inoculant loads are therefore necessary, especially with respect to the severe and dry Indian summers when soil temperatures are high (Table 2). Under these conditions the aim is to achieve as high an inoculant load per seed as possible. In India inoculants are supplied in packets ranging from 100 to 200 g in weight per 'acre-size' packet for small as well as large seeded legumes. This provides higher inoculum rates with allowance for adverse factors like (1) wastage by adherance to mixing vessel and sowing implement; (2) rapid death due to desiccation after application; (3) death due to presence of seed coat toxins; (4) decrease in numbers due to competition from antagonistic microbes; (5) rapid death due to accidental exposure to sunlight and high temperatures and (6) safety against uneven application of inoculant on seeds. Higher quantity of inoculant not only provides wider safety margins but in fact gives better nodulation and yield response if higher amounts are made to stick on the seed coats. (Table 3). When treated with ten times the recommended dose, highest nodule numbers were obtained with different varieties of soybean at Bareilly (Trikha and Nave, 1976).

Table 3. Inoculant quantity requirements to supply 300 cells/seed for some legumes grown in India. *

Seed Type	Av. no. seeds/kg	Normal seeding rate in kg/acre	Grams/acre of inoculant required
Cicer arietinum (Kubuli gram)	3500	40	4
Cicer arietinum (Bengal gram)	7840	45	11
Arachis hypogaea (ground nut)	2400	45	3
Lens esculenta (lentil)	32650	25	24
Pisum sativum (peas)	4010	12	31
Vigna radiata (green gram)	26870	15	12
Vigna mungo (black gram)	20680	15	24
Trifolium alexandrinum (berseem)	39160	10	12
Glycine max (soybean	5840	30	5

* Bactogin Laboratories (unpublished data). Calculations are based on proposed I.S.I. specifications of a minimum of 10^7 viable rhizobia/g inoculant before the expiry period. Inoculants for the above legumes are supplied in 'acre-size' packets of 200 grams.

INDIAN INDUSTRY AND QUALITY CONTROL

In 1970 small scale production of inoculants was being done at six research institutions (located at Delhi, Pantnagar, Ludhiana, Hissar, Jabalpur, Coimbatore) and one commercial unit at Bombay. In 1976 there are more than 40 private and government owned units. Some of the private units have their own research and quality control facilities while others are very small having no research facilities and means for checking the quality of their products. Such a situation is somewhat disturbing since inoculants of poor or indifferent quality are being reported by several workers (Dube, J.N., pers. comm.). The majority of the Indian farmers are yet to be educated for inoculant usage, and in the absence of quality control enforcement there is the danger that inoculation failures due to poor quality could make them skeptical about this input. In U.S.A., official quality control of inoculants was relaxed in the 1930s. By the late 1950s many nodulation failures attributed to poor quality inoculants were noticed. In Australia also poor inoculants were detected and field failures resulted (Date, 1974). These two instances serve to highlight the fact that immediate quality control procedures for ensuring high quality of inoculants are imperative for India as well. Fifteen more state owned units are likely to be opened shortly in different states with a central grant of 1.7 million rupees (0.2 million dollars) (Directorate of Pulses Development, Lucknow, unpublished data). With the coming in of these units the need for quality control will further increase. The I.S.I. has recently constituted a panel of experts to draft Indian inoculant standards (Anonymous, 1976).

Manufacturers in Australia are supplied with selected strains of rhizobium properly screened and serotyped from a central *Rhizobium* bank. Quality enforcement then becomes easier on a national scale. In the U.S.A. private manufacturers have their own rhizobial collections and quality control; official quality control enforcement having been withdrawn some years ago (Burton, 1976, Date, 1974). In India although some of the better private manufacturers are maintaining their own *Rhizobium* collections and quality control as in the American system, they are soon to be brought under quality control of the ISI, a government managed independent body. The Government of India is further contemplating to enforce this through an Act of Parliament making it mandatory for all manufacturers to observe I.S.I. standards.

Most of the units are producing broth by shake culture in 1 to 2 l size glass bottles or flasks. Fermentor facilities exist at the Pantnagar, Coimbatore and Delhi units and as far as is known to this author, only one commercial manufacturer is using fermentors. Apparently fermentor facilities need to be further developed since in large scale productions on shakers quality check on every bottle becomes difficult. Mixing the contents of a 2 l contaminated bottle very often spoils the entire 200 l batch (Sahni, unpublished). Gulati and Seth (1973) improvised a portable fermentor by modifying a small steam sterilizer. Date (1974) has discussed the suitability of small autoclavable galvanised

steel tanks of 50 litres capacity as suitable for Indian conditions. At Bactogin Laboratories, improvised mild steel tanks of 200 l capacity have been successfully used for commercial production over the past several years.

Larger scale production necessitates the use of cheaper culture media or new formulations on which limited information is at present available. Nandi and Sinha (1974) recommend sugar, mannitol and oxoid yeast extract and reported molasses also to be useful. The Rallis unit in Bangalore is using malt extract. Iswaran and Apte (1970) have studied the effects of malt extract on the efficiency of *Rhizobium japonicum*. It has been observed that boiled soybean extract, sugar, and mannitol are superior to yeast extract, sugar, mannitol for culturing *R. japonicum* in fermentor (Bactogin Labs. unpublished data).

For the approximately 30 million hectares (75 million acres) under legume crops, India requires 75 million acre size packets per annum. Statistics of inoculants actually marketed are difficult to obtain but it is estimated that the total number of acre size bacterial inoculants being marketed in the country has not so far exceeded the 1.1 million mark in any single year. Obviously, therefore, India offers considerable scope for further exploitation of the rhizobial symbiotic system through effective legume inoculation.

CONCLUSIONS AND SUGGESTIONS

Apparently, more research data need to be incorporated into production for improving the quality and performance of Indian inoculants. Most of the work is scattered and needs to be centrally pooled for the benefit of inoculant production. For instance, strains of rhizobia used in the several countrywide surveys mentioned earlier and found promising or useful in the trials could be deposited in a centrally maintained *Rhizobium* bank and made available to the manufacturers for the production of inoculants as in Australia. As a result, a lot of labour wasted in duplicated work could be avoided. There is also the need for the systematization of pooled data on Indian rhizobia to enable easier reference and retrieval. There is information available on different host varieties and rhizobial strain symbioses (Sharma and Khurana, 1975; Bapat *et al.*, 1975; Simhadri and Tilak, 1976; Narula *et al.*, 1975; Bhatnagar, 1971; Nagaraja Rao, 1974; Rai *et al.*, 1974) which needs to be exhaustively reviewed for the selection of broader spectrum strains for inoculant production. Information available on the ability of strains to bear edaphic, temperature, moisture, soil competition and antibiotic stresses (Roughley, 1976) is also necessary in such a programme. Information already available with regard to serology (Gaur, 1975; Pal and Bannerji, 1974; Dadarwal and Sen, 1973; Dadarwal *et al.*, 1973, 1974, 1975; Lahiri, 1974); salt tolerance (Pillai and Sen 1966, Pillai and Sen 1973); temperature and moisture stresses (Subba Rao, 1971); pesticide effects Balasubramaniam 1973; Kulkarni *et al.*, 1974; Rai *et al.*, 1975); effects

of associative or antagonistic soil microorganisms (Sethi and Subba Rao, 1969; Jain and Rewari, 1974); antibiotics tolerance (Chopra and Venkataraman, 1969; Wankhede and Bhide, 1972) and weedicides (Gaur and Misra, 1972) have to be further extended to fill the gaps in our knowledge regarding the rhizobia isolated and available in India. The practice of broadcasting seeds of certain legumes like green-gram and black gram under dry, hot and uncertain rainfed conditions, necessitates work on the lines suggested by Brockwell and Phillips (1970) wherein isolated and pelleted seed could be sown with the strong expectation that the inoculant will survive and seedling nodulate.

It would also be worthwhile to investigate how much better sterilized carriers are than unsterilized carriers for the rhizobial strains being used in India (Dart. pers. comm.) Chakrapani and Tilak (1971) have reported that even old expired soybean inoculants of Nitragin Company showing equal amounts of rhizobial and contaminant populations gave excellent nodulation and yield responses. It is possible that not merely the numbers but it is the type of contaminant that affects inoculant quality and performance. Further research on this aspect could save the manufacturer the expense and labour of carrier sterilization in cases where it is not necessary.

Lately a need for working out the associative compatabilities of different rhizobial strains for the same legume, as also for the different *Rhizobium* species for different legumes, in the same inoculant, has been stressed (Tilak, K.V.B.R., pers. comm.). Due to the poorly developed rural transport and communications, the timely distribution of inoculants sometimes becomes very difficult. If intra specific and inter specific combinations could be worked out for the strains already available and these findings incorporated in inoculant production, it would enable the same packet to be supplied for a different crop in the following season (provided this falls within the expiry period), thus reducing returns of unused packets from the previous season. To improve inoculant performance, chemical additives have been tried (Khatri, A.A. pers. comm.). Rai, Shrivastava & Prasad (1975) observed bigger nodules over controls both with and without field yard manure in the case of ST 4 variety *Cicer arietinum*. Addition of coconut water and sucrose resulted in a five time increase in nodule numbers in soybean (Iswaran *et al.*, 1970). The incorporation of charcoal in FYM and in peat has given very good results (Chakrapani and Tilak, 1974). However, this aspect needs exhaustive investigation. Additives like TIBA (Trio-iodobenzoic acid), Lindane, DDT, Cytokinin, Indole acetic-acid, "Amiben" and "Cycocel" were observed to show either deterimental effects or to have no effect on nodulation of soybean when incorporated in the inoculant (Bactogin Labs. unpublished data).

Of all the countries in the world, India grows the greatest variety of legumes. It has 35 per cent of the world area under pulse crops, but the daily per capita availability of these remains a low 50 g against the FAO recommendation of 104 g. The availability of meat and milk is also very low. Evidently, therefore, India offers the greatest scope for increasing legume production through massive inoculation programmes.

Due to historic and geographic reasons the Indian subcontinent is populated by people of different ethnic origins speaking different languages and having regional traditions. Educating the farmer in the use of *Rhizobium* inoculation is fundamental to any legume inoculation programme. However, because of language and other differences, this education has been slow. Better means of extension and education are, therefore, necessary to make legume inoculation a success in India.

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NOTE: The final page of Dr.Sahni's references was misplaced during the production of the Proceedings, and a replacement was not available at publication time. We sincerely regret this ommission.

LEGUME INOCULANTS FOR LATIN AMERICA

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INTRODUCTION

In order to simplify the situation of Latin America let me remind you that the geographical limits are from the south of the U.S.A., the Rio Bravo down to the Antartica. In other words, from the 33° north to the 55° south.

All along this enormous territory there are important chains of mountains (Cordilleras) with elevations of 5,000 to 6,000 m, so that, we have a very heterogeneous land for which to describe the problems of its Legume Bacteriology. There are all kinds of climates: tropical, temperate and cold zones.

Due to these different climates, we have a very many native legumes. Burkart (1952) gives the following list of cultivated species of Latin America origin:

Prosopis juliflora, Inga feuillei, Caesalpinia spinosa, Lupinus mutabilis, Psoralea glandulosa, Arachis hypogaea, Pachyrhizus ahipa, Tipuana tipu, Phaseolus vulgaris, Phaseolus lunatus, Phaseolus caracalla, Erythrina crista-galli, Lonchocarpus utilis, Aematoxylum campechianum, Pachyrhyzus erosus and others, *Pithecellobium dulce, Gleditsia triecanthos, Phaseolus coccineus, Phaseolus acutifolius*. To these, we must add the even longer list of sub-spontaneous and introduced legumes, which have a fundamental economic value for most countries.

Before going into the description of the present state of the Latin American inoculants, one must stress the importance of the following general points:

Meetings of Latin American Legume Bacteriologists

We have had these meetings since 1964, every 2 years. This gathering together of all our work began with the active help of R.A. Date, when he was in Uruguay as expert of the FAO. In synthesis, the story of these meetings shows their importance generally concerned as they were with the practical application of Legume Bacteriology knowledge.

Up to date we have had the following meetings:

1. Montevideo, Uruguay 1964. Participants: 19; Chairman: R.A. Date.
2. Buenos Aires, Argentine 1965. Participants: 27; Chairman: E. Schiel.
3. Concepcion, Chile 1966. Participants: 12; Chairman: L. Longeri.

4. Porto Alegre, Brazil 1968. Participants: 52; Chairman: J.R. Jardim Freire.
5. Rio de Janeiro, Brazil 1970. Participants: 94; Chairman: J. Dobereiner.
6. Montevideo, Uruguay 1972. Participants: 46; Chairman: C. Batthyany.
7. Resistencia, Argentine 1974. Participants: 94; Chairman: P. Fuentes Godo.

   The next one (8) will be this year, in October, at Cali, Colombia; Chairman: P. Graham.

In some of these meetings we had the honor and pleasure of the participation of others such as: J.M. Vincent, F. Bergersen, P.S. Nutman, D.O. Norris, R.A. Date, P. Graham, D. Hubbell. It is obvious that these meetings are of great importance for us.

Committee of Latin American Legume Bacteriologists

We have had this committee since 1968 (Chairman: C. Batthyany). Today, there are 10 countries represented in this committee, with the hope that this list will be soon increased with the inclusion of the 18 missing countries.

According to a personal communication from Vincent, there were 4 recipients of "*Rhizobium* Newsletter", in Latin America in 1961, while in 1975 there were 36 subscribers.

The Latin American Committee has its own "News", published by P. Graham, which keep us up to date with the world literature. The mailing list of our Committee has the file-cards of 150 Latin American Legume Bacteriologists.

Associacao dos Fabricantes de Inoculantes do Brasil

This association started to work in 1975, due to the efforts of Jardim Freire and could be the beginning of a continental level association.

International project of FAO/UNEP

This has just started in Latin America and Africa. Its main object is to establish the production and right use of controlled quality inoculants. The first stage may be completed by the middle of October. The second step will be the organization of demonstrative field trials in the different most important zones. This will be attended to by E. Reynaert for the Soil fertility and Plant Nutrition aspects by J.R. Jardim Freire and C. Batthyany for the Legume Bacteriology part of tropical and temperate legumes.

International project of UNEP/UNESCO/ICRO (pannel on Microbiology)

This is working on the creation of the MIRCENS in Latin America for which we have proposed CIAT at Cali, Colombia and IPAGRO at Porto Alegre, Brasil, as the first.

This communication aims to give you all the information received as to the actual situation of Latin American Legume inoculants. It

will try to give a general view, stressing specially what is still missing and considering possible improvements in the symbiotic fixation of nitrogen.

In order to organize this communication, I shall discuss each country separately within its zone, and will try to arrive at some conclussions.

THE PRESENT STATUS OF THE INOCULANTS OF LATIN AMERICA

North Latin America: Mexico

In a personal communications, Maria Valdes from the Instituto Politecnico Nacional of Mexico gives us the following information:

Mexico is a country where nitrogen fertilizers are neither abundant nor cheap, and which uses many different legumes. The inoculation of seed is very far of being a common practice. In this country, there is a great scepticism concerning the use of inoculants, in part due to inherent problems of the Legume Bacteriology but also because: (1) there is a lack of professionals; (2) the teaching of Soil Microbiology is insufficient; (3) the inoculants are not controlled in quality and (4) the soil ecology and climate are very diverse.

Investigation and organization still need the work of bacteriologists, responsible inoculant industries and agronomists of all different levels. There is planned a real symbiosis between the Institute and the 8 different Government Agricultural Investigation Centers, which have 35 Agronomic Schools spread throughout Mexico.

The most important legume are (1975):

Beans, *(Phaseolus vulgaris)*. Area: 2,200,000 ha; inoculated 40,000ha; (1.8%) This is one of the most important crops, occupying the second place in importance as a basic food and the sixth place in national production value. It is the principal source of proteins for lower income people of this country. Beans have been sown for centuries so, in the majority of the soils there are native rhizobia with a wide spectrum of efficiency.

Soybeans, *(Glycine max)*. Area: 400,000 ha; inoculated 250,000 ha. (62.5%).

(Cicer arietinum). Area: 240,000 ha; inoculated: 25,000 ha, (10.7%)

Lucerne, *(Medicago sativa)*. Area: 180,000 ha; inoculated: 30,000 ha, (16.6%). It was introduced more than 450 years ago, so there is a good number of native strains of *R. meliloti*, in the soil, with different efficiency.

Other important legumes are: *Lens culinaris; Vicia faba; Phaseolus lunatus; Coccineus phaseolus* and *C. acutifolius; Leucaena leucocephala;*

Clitoria ternatea; Stylosanthes gracilis and *S. humilis*. The most important tropical legumes studied in Mexico are: *Macroptilium atropurpureum, Desmodium intortum, Centrosema pubescens, Glycine javanica* and *Pueraria phaseoloides*.

As to inoculants, Valdes informs me that there are two national factories, but they make inoculants for only non-tropical legumes. Those for the tropicals are imported from the U.S.A.

The first work on Legume Bacteriology in Mexico was by Dr. C. Casas Campillo in 1944 at the Instituto Politecnico Nacional.

To this important information of M. Valdes, I can add that this exposition was begun with Mexico, not only as the most northern country of Latin America, but also because it is typical of the problems of Latin American Legume Bacteriology. We are still in need of basic knowledge in zones with very different ecologic factors, we need professionals, extension work and the improvement of teaching. Mexico entered our Legume Bacteriologists' Committee in 1972, and an effort is being made to improve the situation. Post-graduate courses, field trials with demonstrations and other activities have been organised which have already begun to show benefits at all levels. Members of the Valdes group have received post-graduate training in: Colombia, U.S.A., Brazil; they have participated at international congresses and visited the most important centers in Australia.

From all this, we conclude that although many things are yet needed, Legume Bacteriology in Mexico is improving steadily.

Central and Caribbean Latin America

We do not have any information about the Legume Bacteriology of the countries of these zones.

From Panama there is an UNDP (1973) informing about some improvement of pastures where native legumes are well represented and, in general well nodulated. Native strains for the cowpea group were specially abundant and generally all the trials with legumes were done without inoculation except *Lotononis*. Concerning the future of Legume Bacteriology, there is a lack of projects. Norris (1969) concluded from his investigations in Panama that inoculation is of little value, except when there is a high specificity between host and strain.

In Costa Rica, there is actually a post-graduate course, (D. Hubbell, from Florida); therefore, we hope to obtain some information of this country.

We should take into account that the seven Latin American meetings took place in the southern part of the continent. The first northern meeting will be this year when we hope that we will have the pleasure of incorporating several countries of this zone in our Committee.

Tropical Latin America

Just as from the central zone, we do not have information from

Venezuela, Bolivia, Guayanas.

Colombia

The Colombian's Legume Bacteriology Center, is the Centro Internacional de Agricultura Tropical (CIAT), placed at Cali, where P.H. Graham has been working for several years. In a recent personal communication he informs that in the future the Center will work with 3 Ph.D. microbiologists; one of them will work on legume pastures, with *Stylosanthes*, *Desmodium* and *Centrosema*; while the other two will continue the work with *Phaseolus vulgaris*. With pastures, there will be a lot of work with strain selection and host specificity. In the work with *Phaseolus*, they will concentrate on more varieties than with strain selection. They will also try different inoculant carriers.

In Colombia, the only quality inoculant producer is the CIAT, dealing with orders up to 300 Kg. of peat. The strains are controlled by the CIAT, or they come well studied from other centers. The requirement is more than 10^8 rhizobia/g. Demand is principally for soybean inoculant. The CIAT is not interested in industrial production of inoculants.

CIAT has the facility of teaching at post-graduate levels and this is already being noted on neighbour countries, with corresponding benefits. Mexico, Guatemala, Ecuador, etc. have already had their Legume Bacteriologists trained at the CIAT.

Peru

In a personal communication, Vallenas from the Universidad Nacional de La Molina informs that although there are national inoculants for medics and clover, the most important is Nitragin, from the U.S.A.

All the soybean is inoculated, peanuts about 30%. Beans, in general, are not always inoculated because they do not give good results. Vallenas estimates a total of 170,000 ha of large grain legumes.

Mackie from Universidad de Ayacucho reports on his own small scale inoculant production, and that they are just starting a Legume Bacteriology Laboratory at San Marcos University, where the technicians receive training from Graham of Cali. The country does not have any legal regulation for inoculant control.

Ecuador

Although we are in contact with some Legume Bacteriologists of this country, we have too little information for an exposition. There is an agricultural investigation center (INIAP), which will be the place for our future efforts in this subject.

Tropical and Temperate Latin America: Brazil

This country is named in nearly all lists in the tropical Latin American zone, but for this subject, it will be considered a separate zone, due to its size and importance.

It is very well known how Brazil has developed recently. Likewise, we also see the improvement in Legume Bacteriology, with a continuous increase of well directed efforts, and with very clear objectives. In 1970, there were five official institutes making inoculants; most on a small scale, on agar, and two private firms of peat inoculants. (Batthyany 1970).

Instituto de Pesquisas Agronomicas (IPAGRO).

Jardim Freire, director of the official laboratory (Secretaria de Agricultura, Porto Alegre), informs me that they began the work in IPAGRO (Instituto de Pesquisas Agronomicas), in 1950. The lines of this activity were always; (1) improvement of inoculants and N-fixation in legumes with investigation applied to resolve problems; (2) inoculant control and: (3) promotion of inoculant use. IPACRO has developed along the following lines:

(1) selection of effective strains for soybean, clover, lucerne, lotus, beans, vetches, etc., the maintenance of the collection and the distribution of the recommended strains to the Laboratories. The actual collection (1975) consists of:

	No. of strains
R. meliloti	42
R. trifolii	114
R. leguminosarum	90
R. phaseoli	95
R. japonicum	117
R. lupini	23
Rhizobium sp. (cowpea)	113
Rhizobium sp. (lotus)	38
Total:	532

(2) Limiting soil factors specially P deficiency, Al and Mn toxicity.

(3) Inoculants production and technology In 1950 they began with agar culture and in 1954 with peat as carrier. In 1956 began the first private industrial production.

(4) Quality control The responsible technicians of the four most important manufacturers of Brazilian inoculants were advised and trained by the IPAGRO Laboratory Center. There has been informal control for years. In 1975 the Government legalized the obligatory control, which is now in its beginning.

(5) Extension The permanent object of IPAGRO is the promotion for use and publication of technical results at all levels, field demonstrations, meetings, small courses, booklets, etc. Now there are 5 private inoculant industries, all of them on peat base, partially or non-sterilized. Last year, Brazil had 8 million ha of soybean. From this total, 40 to 50% was sown with inoculated seeds. In 1975 they established the Asociacion de Productores de Inoculantes de Brasil, from which we hope to have a new impulse to progress, specially for more extension and better inoculant quality.

Other centres

Another very important legume Bacteriology Center is the IPEACS, near Rio de Janeiro, directed by Johanna Dobereiner, and the Instituto Agronomico de Campinas, under the direction of E.S. Lopes. This last center, where Dr. Norris worked for 2 years, is specialized in tropical legumes. The IPEACS Center in Rio is also working in the biological N. fixation with other plants; its very important results makes it one of the world centers investigating this aspect of the biological fixation.

All the three mentioned centers have the facility of training post-graduate students, which are needed in other zones of the country.

Temperate Latin America

Argentine

The information given below is a resume of a personal communication from Rosa Dieguez, of the Instituto Nacional de Tecnologia Agropecuaria (INTA). National and foreign inoculants are used in Argentina.

Among the national ones there are private and official Institutes.

Private Institutes

Agricultores Federados Argentinos (Ag. Casilda F.L. Beltrán 2255, Prov. de Santa Fe). This inoculant is on agar(1) - Nitrobac (Bogotá 258, 2° p. Buenos Aires). Inoculant with peat carrier(2) - Radibac (José P. Tamborini 3094). Inoculant on vegetable carbon. This firm previously used Hyperphosphate as carrier(3).

Official inoculants

Estación Experimental Agrícola de Tucumán (C.C. 75, Tucumán Prov. de Tucumán); inoculant impregnated on waste-pulp of sugar cane(4) - Instituto Agrotécnico (Las Heras 727, Resistencia, Prov. de Chaco); on peat carrier(5) - Laboratorios de Bioedefología (R.A.G. Blvd. Pellegrini 7100 Sta. Fe); inoculant on vermiculite(6) - Unidad Simbiosis INTA (C.C. 25 Castelar, Prov. de Buenos Aires); inoculant on agar(7)

The imported inoculants are:

C.K.C. inoculant on peat basis from Patrick, U.S.A. Crowford Keen Co. (San Martin 232, Bs.As.)(8) - Nitrasoil, Uruguay; inoculant on peat basis with more than 100 x 10^6 R/g. (Nitrasoil, Florida 622 Pso.4 Of. 17, Bs.As.)(9) - Nitragin from U.S.A., on peat (Address: See No. 1)(10) - Terrasoja from Germany (Visscher, Corrientes 538, Bs.As.); on peat(11) - Nitrur, Uruguay on peat basis with more than 100 x 10^6 R/g (12) At present there are no obligatory controls in Argentine. There are projects at the official level to establish integral controls shortly.

The most important legumes (1974-75) are:

Lucerne	2,500,000 ha	Peas	30,000 ha
Chick-peas	8,000 "	Lentils	8,500 "
Beans	150,000 "	Peanuts	400,000 "
Soybean	400,000 "		

We do not have information on inoculation percentage.

My informant states the absolute lack of professionals as the most important problem of the Legume Bacteriology in Argentine. This is particularly serious in the case of agronomists. The number of technicians is not enough to cover the range of climate and soils in the Country; for that reason information is still lacking many areas.

Another important problem is the lack of inoculant control. Early legal regulation would reduce problems of the use of uncontrolled strains, low concentration R/g. bad carrier etc.

The product Nitrasoil (Address of inoculant No. 9), will be manufactured also in Argentine on peat basis to provide more than 100 x 10^6 R/gr. This will be in a new factory with a wide industrial production capacity.

Legume Bacteriology began in Argentina in the 30's, and little by little became concentrated in INTA. In this Center they work on almost every aspect of the subject. There is a lot of work on strain selection and maintenance, inoculant carriers, herbicides and biocides, etc. (Batthyany 1970). Another important investigation center is: La Universidad de La Plata and Resistencia.

Chile

The Legume Bacteriology Center in Chile is in the Universidad de Concepción. Since 1969 they have their own peat-based inoculant (Nitrofix) with a strict control of quality- with viable plate and plant counts using Australian standards. We do not have information about the extent of legume crops, or inoculation percentage.

Paraguay

There is no information. Inoculants are imported from U.S.A., Uruguay and Brazil.

Uruguay

For various reasons this is the Country of Latin America where the Legume Bacteriology reaches a high level. Uruguay has a total extension of no more than 16 million ha, all of them under similar ecologic condition. An exclusively pastoral country, its economy depends basically on pastures and agriculture. The improvement of pastures and legume crops, was in the past and is still the vital point for the economy of the country.

Legume Bacteriology began in 1951, in a private firm, Laboratorios Dispert S.A., making the first tests of native strains, and others obtained from foreign collections. In 1952, this firm made a liquid inoculant, which afterwards changed to inoculant on agar (1960). With the increase of pastures, the observations of faults with inoculation increased and the importance of the problem caused the Government to ask for an expert from FAO. From 1962 to 1964, R.A. Date organized and directed the official Laboratory and instructed Laboratorios Dispert S.A. on the production of an inoculant on peat basis, which was marketed in 1963. (Date 1965).

The official Laboratory established quality levels, like the standards of Australia. It works with seed pelleting, selection of strains at lightroom, green house and field level, survival of rhizobia in different peats, etc. As already mentioned, the first Latin American *Rhizobium* Meeting was organized by this Center.

In Uruguay: (1) All the legumes' seeds are inoculated at sowing; (2) quality of inoculants is regulated by law. Today there are two inoculants: Nitrasoil (since 1963) and Nitrur (since 1965). Inoculants are no longer imported. The country goes on sowing legumes, so that the technical aspects are still very important. The economically most important species are: clover (white, red and subterranean), lucerne, lotus, soybean. There has been a marked tendency in quality improvement of our inoculants, as you can see in the following table:

Percentage of Batches with Different R/G (x 10^6) *

Year	Less than 1	1-100	100-500	More than 500	More than 1000
1964	0	56,6	35,6	6,6	6,6
1965	0	33,3	33,3	33,3	9,5
1966	18	63,6	18,1	0	0
1967	16,6	33,3	38,8	11,1	11,1
1968	6,0	36,3	42,3	15,3	6,0
1969	11,1	14,8	37	37	33,3
1970	0	14,4	0	85,3	61,9
1971	0	11,1	22,2	66,6	50
1972	0	11,1	11,1	77,7	72,2
1973	4,3	0	0	95,6	73,9

* viable count with plant dilution test.

Nobody can doubt the importance of control. From this table we can also see that in columns: Less than 1 and 1-100, there is always a small number of batches. This confirm that the strict control of production will avoid failures, which may discredit all our work.

CONCLUSIONS AND RECOMMENDATIONS

Vincent (1970a) states that: "There needs to be the utmost co-operation between the agronomist concerned with legume use and the microbiologist concerned with looking after the rhizobial side. This is true whether we consider the question of evidence of a need for legume seed inoculation or whether we are concerned with cases of failure that might be attributable to the inoculum or how it was used..."

Recognition of the need for legume inoculation is particularly likely to depend on a sufficient awareness of the potential significance of the rhizobial factor. It requires attitudes of mind that will stimulate the agronomist to look below the surface and encourage the

microbiologist to get out into the field so that together the situation can be more expertly evaluated".

We know the order of our duties (Vincent 1970b). The most important points of our work are: (1) selection of strains; (2) control of traditional and introduced legumes' yields, which includes investigation of necessity for inoculation; (3) production and control of quality inoculant; (4) adequate use of inoculants.

Although the importance of each of these points is obvious, we see that in Latin America they are still not well handled. Even in Uruguay and Brazil, we still see cases where legume introduction is done neglecting or paying scant attention to the *Rhizobium* side.

In this connection, it is very important to emphasize that Latin America needs the training of new professionals, in other words, the teaching at all levels. This is valid for the different Universities (Agronomists, Chemistry, Veterinary and Medical), all different agricultural schools and even more at the farmer's level. We have Legume Bacteriologists from all the different Faculties (Medical, Veterinary, Biochemist, etc.) and we applaud if we find one who is an "Agronomist".

The most important part of Latin America belongs to the tropics, where our subject is even more difficult and poses more problems (Graham and Hubbell 1975). But even for temperate zones, there are continuous and serious problems. In order to refer strictly to Latin America inoculants, I would not like someone to ask how many doubtful or really bad inoculants are actually in use.

Maybe we could define the most efficient steps for progress, as follows: (1) one official center attending to the basic investigations such as selection of strains, recommendation of legume species and help in establishing inoculant industries, (in most cases preferably private); (2) as soon as an inoculant has been approved for the zone, the official center and the fabricant(s) begin publication and extension work, to diffuse the results obtained. Something like this has happened in Australia (Date 1969) and in Brazil and Uruguay.

This communication is full of gaps in information, but we think that this very absence of data indicates our urgent obligation to act. We are optimistic and hope that our already existing centers, with the help of different international projects, will soon change the situation and will assure widely the right production and use of inoculants wheresoever legumes are sown.

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PROBLEMS IN DEVELOPING AN INOCULANT CAPABILITY

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INTRODUCTION

The critical fertilizer situation during 1973 - 74 in terms of increased prices and unpredictable availability had a serious impact on production of food crops in Guyana and undoubtedly in most developing countries which lacked an indigenous source of fertilizer supply. Many farmers were reluctant to pay for the increased cost of fertilizer inputs thus affecting production considerably. The high prices and unpredictable availability of fertilizer import needs are likely to prevail for some time, thus making it mandatory for us to find ways and means to satisfy at least some of the fertilizer needs. To satisfy the nitrogen needs of farmers and commercial state farms engaged in legume production, a programme for the development of a pilot plant unit for the production of legume inoculants was undertaken by the Ministry of Agriculture in Guyana. I am happy to see that this programme for legume inoculant production is being given the special emphasis it deserves.

Guyana in collaboration with other Caricom countries has recently embarked upon a programme for the large scale production of several grain legume crops to satisfy the protein and food requirements of the people in the Caribbean countries. Such a programme could only benefit from the establishment of a national inoculant production system. In the past we relied upon the commercial companies of the more advanced countries for the supply of our legume inoculant needs. The use of imported inoculants resulted in frequent nodulation failures. These inoculants were no doubt supplied in good faith and the number of factors involved make it difficult to determine the exact reason for failure. Very often however, the numbers of rhizobia were found to be too low to effect proper nodulation especially in the very infertile soils where the rhizobial population was nonexistent or very low. Our observations would seem to suggest that the improper storage conditions during transit could be a major limiting factor.

In Guyana we are in the process of establishing a pilot plant production unit. In this presentation an attempt is made to outline some of the basic requirements for and problems associated with the establishment and operation of such a production system. This report however is biased towards the situation in Guyana as experienced by the author.

REQUIREMENTS FOR INOCULANT PRODUCTION

Laboratory and Transfer Room

Certain basic requirements are essential to the development of an inoculant capability. One of the basic requirements is a well equipped laboratory for bacteriological work. This forms an essential component in the production of quality legume inoculants. The laboratory must be provided with adequate facilities and equipment for doing basic research and development work on Rhizobia. Some of the basic research and development work consist of maintenance of a rhizobial culture collection; isolation and screening of rhizobial strains; nitrogen fixation studies, serology and general mission-oriented research.

A serious problem common to most countries in the tropics is contamination by fungal spores. It is essential therefore that a transfer room containing a laminar flow cabinet be incorporated in the laboratory. Also the installation of ultra-violet light will greatly reduce the risks of contamination.

Fermenter

The production of rhizobial cultures on a large scale involves the use of stainless steel fermenter vessels with adequate facilities for aeration and agitation of the culture medium. The size of the fermenter vessel is determined by the required scale of production, but for a pilot plant the size ranges from fifty to three hundred gallons.

There is no such thing as an 'ideal' fermenter design. Each one has its own advantages and disadvantages. Generally the continuous culture production system is undesirable for rhizobial production because of the long generation times of rhizobia and also the difficulties in operating a continuous system aseptically. It is important that a high degree of sanitation prevail to reduce the risks of contamination. This is especially so during inoculation of the fermenter tank.

Pressurized Steam

A source of pressurized steam is required for sterilization of the fermenter tank. The capacity of the boiler or steam generator is determined by the number and size of tanks used.

A source of supply of sterilized air is also needed for aeration of culture in tanks.

Culture Media

The composition of the culture media for culturing rhizobia may vary according to the nutritional requirements of the selected strain. In general the media consist of a suitable carbohydrate, nitrogenous

compound and a source of growth factors.

Different types of culture media are used in the commercial production of rhizobia (Burton, 1965). It is important however to use the media that would give the maximum number of rhizobia per ml.

In our laboratory we have found that with many of the rhizobial strains used in inoculants a mixture of carbohydrates, usually mannitol and sucrose, gives an increased concentration of cells compared to that obtained with a single carbohydrate source is used. Since rhizobia strains differ in their abilities to utilise carbohydrates (Graham and Parker 1964) it is desirable that selected strains for inoculant use be evaluated for 'maximum utilisation' of carbohydrate supply. In commercial production of these strains the most economic medium is used since the economics of production must be considered.

RHIZOBIUM CARRIER

The availability of an inexpensive and suitable carrier for rhizobia is essential for the economic production of legume inoculants. The suitability of any material used as carrier is determined by its capacity to retain the viability of the added rhizobia over long periods without loss of infectiveness and effectiveness. The carrier most commonly used in inoculant industry is peat. Since the composition of peat varies with location of deposits it is desirable that the suitability as a carrier be ascertained before use in production.

In Guyana extensive deposits of peat exist with some of these very close to the inoculant production site. Peat collected during the dry season is dried to about 10% moisture after the excess moisture is allowed to drain off. It is then ground to about 200 mesh before neutralising with finely ground carbonate. The problem of dust is reduced by the use of mineral oil.

PROBLEMS ASSOCIATED WITH ESTABLISHMENT

The problems associated with the successful implementation of a legume inoculant production system are many and complex. A feature common to most developing nations is limited resources both in terms of finance and technical skills. The introduction of a new technology requires adequate supplies of finance and technical expertise in order to achieve any measure of success in increased productivity. The constraints to increased productivity as imposed by such limited resources have been recognized. The situation therefore demands our greatest ingenuity and dedication in order to succeed in our task.

The major problems associated with the successful development of the inoculant production unit in Guyana are: (1) lack of adequate financial resources necessary for the acquisition of costly inputs; (2) lack of technical expertise. One of the major constraints in developing our production system is the high cost of machinery and equipment inputs. Most of the inputs are unavailable locally and as a

result we are almost entirely dependent upon foreign companies for the supply of our needs. It becomes necessary therefore that serious consideration be given to the proper selection of inputs. This is based on the needs and financial resources available. Recently we have experienced considerable difficulties in the procurement of certain types of equipment used in the production process. Another major setback is the delivery time of purchased items. We have had occasions when it took more than one year to receive ordered items. Such difficulties can only contribute to slow progress.

The second and perhaps equally important constraint to production is the lack of technical expertise. The scarcity of personnel with sufficient training and experience poses serious limitations to effective implementation. In overcoming some of the technical problems including equipment design we are greatful for the assistance received from the technical advisors who were associated with INSTOY - Guyana programme two years ago. We in Guyana are especially greatful to Dr. F. Davidson and Dr. L. Frederick for their contribution to our legume programme.

INOCULUM IMPROVEMENT

Rhizobial strain selection for use in inoculant is based on several characters. Some of the characters suggested (Burton 1975; Roughley 1970; Brockwell, J. *et al.*, 1968) as desirable for strain selection include:

1. Nitrogen fixing effectiveness with a specific legume host plant;
2. Ability to nodulate appropriate host in soils containing ineffective rhizobial strains;
3. Ability to survive under prevailing soil and climate;
4. Ability to compete with natural soil microflora.

The process of strain selection by inoculant manufacturers is tedious but essential requirement for successful inoculant production.

The selection of strains on the basis of increased nitrogen fixing effectiveness may increase legume yields in soils devoid rhizobia. However variations in effectiveness of introduced strains in soils containing native strains have been reported (Abel and Erdman 1964; Caldwell and Vest 1970; Ham *et al.*, 1971; Johnson and Means 1964). Such differences in effectiveness could probably be due to competition with the native strains for nodule site and/or to unfavourable soil conditions for the introduced strains. For introduced strains, that are effective nitrogen fixers, to compete successfully with the native strains, they must be introduced at an increased rate (Weaver and Frederick 1974; Holland 1970).

The competitive ability of introduced strains is also influenced by soil types (Damirgi *et al.*, 1967).

The above consideration would seem to indicate that the competitive ability of introduced strains varies with the physical, chemical and biological properties of the soil. It becomes important therefore that some serious consideration be given to the selection of strains from

native populations or from original natural habitat.

Selection of strains on a broader scope and the application of these by improved methods of inoculation may well be the simplest and most economical way of increasing productivity.

Since the nitrogen fixing effectiveness of a particular strain is influenced by the host plant production capabilities and the conditions under which the symbiosis operates the selection of strains for inoculant use would depend on a closer working association among the legume bacteriologist, plant breeder and the agronomist.

Inoculum improvement by the selection of more efficient strains is only part of the problem of inoculant manufacturers. Consideration needs to be given to the improvement of methods of application. There are many situations where the conventional method of application of rhizobia to the seed may be inefficient (Scudder 1975). For example rhizobia adhering to the surface of the seed may be lifted with the seed coat out of the ground or close to the soil surface thus reducing the infection process. Also exposure of the rhizobia to sunlight and high temperature considerably reduces their viability.

Several types of adhesive have been evaluated for their suitability to increase adherence of the inoculum on to the seed. However the main purpose of using adhesive concerns pelleting. This is a technique used to provide a microclimate suitable to the survival of the applied inoculum under unfavourable conditions. Several types of pelleting materials were investigated (Bergersen *et al.*, 1958; Norris 1958; Loneragan *et al.*, 1955; Date and Cornish 1968). Some success have been obtained by pelleting. It has been suggested (Brockwell and Roughley, 1967) that the peat component could be the contributing factor.

Since several factors are involved in the success or failure of pelleting it is important that the technique be evaluated for the individual legume and rhizobia before implementation.

SUMMARY AND CONCLUSION

The major constraints to development of a legume inoculant production system in Guyana are the unavailability of adequate finance and technical expertise.

The production of high quality inoculants depend upon the availability of adequate laboratory and supporting facilities. In the procurement of many of the equipment necessary for production considerable difficulties have been experienced. The need for assistance in the initial establishment and operation depends on help from technical expertise from the more developed world.

In terms of production of high quality inoculants it is important that quality control tests be carried out at all stages in production. The improvement of inoculants relies on the selection of strains on a much broader scope to include soil conditions in addition to developing better techniques for the application of the inoculum. The need for a much closer working relationship among the legume bacteriologist, agronomist and geneticist to find simple solutions to the many problems is essential.

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QUALITY CONTROL OF INOCULANTS

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INTRODUCTION

When the benefit to be derived from the nodulated legume depends on successful inoculation with a *rhizobium*, then it is essential that the inoculant carry the right kind and sufficient number of specific bacteria to achieve that objective. This expectation needs to be realisable under a practical degree of stress (condition of soil, climate and in the presence of antagonists and competitors), not merely under less rigorous greenhouse conditions. A large part of rhizobial research has had the achievement of this objective as its main purpose; this effort can be entirely wasted, and the process of legume inoculant discredited, if the inoculant sold to the farmer is unable to measure up to such practical requirements.

Attempts at legume inoculation followed soon after the nature of the symbiosis had been established by Hellriegel and Wihlfarth in 1888 and the causal bacterium isolated by Beijerinck, the same year. The first method took the form of a massive application of soil from a previously cropped area, but progressed, after a good many failures, to pure culture preparations on agar media and then to the more convenient *rhizobium*-enriched carrier (notably peat), which is largely the culture as we know it to-day.

Early disappointments and difficulties have commonly been due to a failure of technical competence and integrity to match commercial enthusiasm. These early days of legume inoculation are well documented in the classical publication by the Wisconsin group (Fred, Baldwin and McCoy, 1932) who themselves did so much to systematise and build up our knowledge of these questions. They too had a clear perception of what was needed in a good inoculant. The requirements they stated still hold good, and can be paraphrased:

(1) The organism should be proper for the legume, and of only one kind.
(2) The strain should be selected and maintained so as to be effective with the specified legume.
(3) The culture as used should provide sufficient young, active rhizobia - probably several thousand per seed.

Unfortunately these wholly admirable objectives have often not

been realised in practice. In the country of their origin legal provisions were vague and discrepant from state to state. When cultures were checked, the testing procedure and standards were so lenient as to permit any but the very poor to pass. Such progress as was made was due to the technological capacity and integrity of the better manufacturers but it has always been, and is still possible, for a poor quality (or even useless) product to get by under the protection of low standard infrequent testing and the cover-up provided by the common occurrence of adequate or passable rhizobia in many soils of the North American Continent. Lately these "Safeguards" have been added to by the exercise of legal muscle to suppress justified criticism.

AUSTRALIAN EXPERIENCE

On the Australian scene we have been fortunate in that as inoculant production moved from direct government supply to private industry, the condition of its use made cases of inferior quality immediately obvious. We were also lucky that the industry was small enough, and liason amongst departmental, university and manufacturing personnel close enough, to permit the rapid development of acceptable procedures for the improvement and control of inoculant quality. Voluntary acceptance of controls, associated with packet certification of tested and approved cultures, has avoided the snares of legal enactments, with all their difficulties in application to such material.

The account which follows will be almost entirely based on our experience in Australia, the first ten years of which have been documented by Date (1969) and some more recent aspects dealt with in Vol. 7 of the IBP series (Date, 1976; Roughley, 1976).

Establishment of Control and Advisory Body

The transfer of responsibility for the production of legume inoculants based on peat carrier was associated with cases of failure in field use because so many were being applied in areas where their successful use was vital to the operation, and because agronomists and farmers were more than usually aware of its importance.

The organisation known as U-DALS (University-Department of Agriculture Laboratory Service), later (1970) to become the Australian Inoculant Research and Control Service (AIRCS), grew out of these needs and became responsible for: (1) testing, selecting and maintaining strains, and the collection of data from other official groups; (2) the control of inoculant quality; (3) advice to manufacturers, distributors and users; (4) the investigation of problems directly concerned with the manufacture and use of inoculants.

The Work of U-DALS.

Strain selection

Cooperative testing and collation of data led to progressive introduction of strains to replace some which had become less effective or because the new strains were more suited to particular host or field conditions. (Date, 1969, figs. 1-2 and pp 28-32).

Maintenance and issue of mother cultures

Maintenance of cultures was based on lyophilisation and a scheme developed to minimise the opportunity for variation or replacement between the time of plant test and utilisation in manufacture. The detail of the U-DALS procedure is shown diagrammatically in Fig. 3 of Date's article.

Tests of cultures during and after manufacture

The first testing programme was designed to ensure the early detection of any faults and to assist in their avoidance. They were aimed at the broth stage, the freshly manufactured peat and the culture as sold to the farmer. Most of the tests have stood the test of time; some were more useful during the developmental stage and are less obligatory than others. They are listed in Table 1 and have already been described in some detail (Date, 1969; Vincent, 1970). They will therefore be dealt with quite briefly in this account.

Experience of U-DALS over the first 10 years

The early difficulties involved loss of effectiveness of invasiveness in some strains, failure to obtain sufficient growth and frequent overgrowth by contaminants in the broth stage of manufacture. These difficulties were largely overcome within a couple of years, and residual unsatisfactory material detected and rejected. Most of the broths classed as satisfactory exceeded 10^9 viable specific rhizobia/ml.

As broth cultures were improved so were the peats to the point that better than 80% of those manufactured were classed as satisfactory ($> 10^8$/g) and about half of these were at least as high as 10^9/g.

Improvement in cultures at the retail stage took a little longer and depended on pin-pointing difficulties of storage and distribution. It then followed the same trend as the manufactured peat so that almost all would have provided at least 300 rhizobia per seed and with many the figure would have exceeded 30,000.

Data for this early period have been summarised (fig. 4-6, Date, 1969).

Later Difficulties

The work of U-DALS and of its successor organisation (AIRCS) was not without its difficulties. The incidence of these, and their early detection and remedy, provide additional evidence for the value of such regular control surveillance. In its absence faulty cultures would

have continued to be produced and sold, without detection, as they probably are in other circumstances. It is worthwhile looking at these difficulties.

Lost symbiotic capacity

This was encountered early in our experience with lost invasiveness in a culture of *R. trifolii* being distributed by a government agency, and in several other cases, lost effectiveness. We have since had several reminders of the same hazard (Herridge & Roughley, 1976; Labandera & Vincent, 1975; Roughley, 1976).

Poor growth and survival in peat

Several difficulties have been encountered with unpredictable variability in the suitability of peat (even from the same general area); toxicity in overheated peat; death of rhizobia due to "heat of wetting" of overdried peat; and with toxicity due to accumulated salt. These factors, together with evidence as to the value of using sterilised peat and the influence of storage temperature, moisture and access of air, have been well documented in a series of papers (Roughley & Vincent, 1967; Roughley, 1968; Steinborn & Roughley, 1975).

The Question of Standards

There is ample evidence that the minimal number of rhizobia required to obtain full nodulation can range from a few per seed to many thousands according to: method of application of inoculum, temperature and time between inoculation and seeding, toxic factors associated with the seed coat (either naturally or as a result of pesticide application), soil conditions and the competition offered by any rhizobia already in the soil. Our experience has led us to look to 300-3000 per seed as a minimal practical figure, at the same time as we have encouraged the production of inoculants able to better that figure by a factor of 10. The relationship between number in the applied peat culture and that per seed varies a good deal according to seed size and the quantity of seed used with a given volume of the peat slurry; the following are approximate equivalences in the case of seed like siratro (Vincent, (1970) p.128).

Viable rhizobia per g peat	Approximate number/seed	
10^6	50	
10^7	500	Retail minimum
10^8	5,000	Manufactured minimum
10^9	50,000	

TESTS USED FOR THE CONTROL AND EVALUATION OF LEGUME INOCULANTS (Table 1).

From the experience that has been gained in some 20 years

association with the legume inoculant industry, I would like therefore to set out briefly the kind of control programme which seems necessary to ensure the farmer gets to using a good quality product.

Selection of Rhizobial Strain

The finished culture will be no better than the strain(s) from which it is manufactured so that this step is basic to the whole operation. Commonly it will require preliminary screening of many strains in small assemblies followed by more rigorous, and more costly, field testing. Strains already in use will need to be included in such a testing programme.

The selection of a rhizobial strain for use in commercial cultures can be a matter of rather fine judgment in balancing any advantage due to superior performance with particular hosts and/or environment against that associated with a "wider spectrum" strain of good, but less excellent, performance with the general range of hosts and in many situations (Date, 1976 for further discussion).

Who then is to make the decision as to what strain(s) will be used and when a change is to be made? In some countries it rests with the manufacturer who may or may not be well equipped to test strains, or evaluate data. Besides the question of the validity or otherwise of a private independent decision of this nature, such a procedure does not lend itself to ready control or records of results of strains under practical conditions. At the least, it ought to be required that strains be adequately recorded, characterised and approved and that a reference culture be lodged with a controlling authority. Alternatively, and more satisfactorily, the recommendation for strain use should come from an independent body charged with arriving at this decision on the basis of adequate data and after discussion with collaborating investigators. Such recommendations should be uniform for widespread regions and as many host species and cultivars as possible.

Changes in strain recommendations should be undertaken only when there is sufficient positive evidence to justify the consequent inconvenience. Any substitution should be with a fully tested strain taken from a reserve maintained with a background of regular comparisons including field tests over at least two seasons.

Strain Maintenance

Proper identification and recording of strains is essential if useful exchange of information is to be maximised and waste of effort kept to a minimum. Besides this there is no doubt that the proper maintenance of cultures, free of contamination or accidental substitution and genetically stable, is of very great importance (Vincent, 1970 pp 9-13).

Again we have to consider whose is the responsibility. As before the extent to which this might be left with the manufacturer will depend

Table 1. Tests Used in the Control of Legume Inoculants.

Test	Purpose
I. STRAINS (Vincent, 1970; pp. 74-104; Date, 1976).	
1. Screening (Lightroom & greenhouse)	Preliminary strain selection; regular check of current strains.
2. Field tests	Basis of full strain test; selection of current and reserve strains.
3. Maintenance (Vincent, 1970, pp 9-13; Date, 1969)	
(a) Long term	Backstop reserve cultures (central collection.
(b) Short term	Supply of authentic mother cultures.
4. Qualitative-quantitative check of symbiotic capacity (Labandera & Vincent, 1975)	Safeguard against overgrowth with non-invasive or ineffective mutants.
II. BROTH CULTURE (Date, 1969; Vincent, 1970 pp 122-123)	
1. Turbidity	Estimate of total growth (viable & non-viable).
2. Total cell (microscopic count)	Total growth (detection of contamination).
3. Gram-stained smear	Detection of contamination
4. pH	Detection of contamination
5. Viable (plate) count	Major quantitative criterion: detection of contamination, with (2), indication of faulty production.
6. Agglutination*	Detection of wrong strain and/or gross contamination.
7. Inoculation of glucose peptone agar*	Detection of contamination
8. Plant test (10^7-10^{10})*	Check on retention of symbiotic capacity.
III. PEAT CULTURES (Vincent, 1970 pp 123-125).	
(a) Sterile peat	
1. Viable (plate) count	Major quantitative criterion & check on purity; possibly back up with serology - for strain identity.
2. Plant count*	Strain authenticity and symbiotic stability.
(b) Non-sterile peat	
1. Plate count*	Usable with better cultures; possible serological back-up.
2. Plant count	Major quantitative criterion, check on strain authenticity and symbiotic stability.

* Optional

on his available laboratory facilities and technological capacity. With smaller manufacturers and during an industry's development, it would seem essential that this aspect too be in the hands of a central competent authority at least as a back-up, if not for total continued provision of authentic mother cultures.

Control that needs to be exercised at this stage should include discriminating quantitative-qualitative plant tests able to detect the emergence of an undesirable mutant (Labandere & Vincent, 1975). Such regular tests need to be coupled with a programme aimed at minimising opportunity for variation, strain substitution or contamination between the time of the most recent plant test and the commercial use of the strain. This was the aim of the U-DALS programme for the provision of mother cultures on a monthly basis to manufacturers. (Date, 1969).

Tests with Broth Culture

These are designed to detect significant contamination at this stage and to determine the number of viable specific rhizobia. The relative significance of those two faults will depend on whether the broth is to be used to establish a predominantly rhizobial population in a non-sterile peat (when viable number of rhizobia will be more important than a slight degree of contamination) or to seed a sterile peat (in which a smaller initial count could still result in a high final figure, but any contamination could undo the advantage of prior sterilisation).

Tests primarily designed to test rhizobial numbers are turbidity and microscopic count (for an immediate check on total growth), and plate count (for viable numbers). The two counting methods may also reveal contaminants which can also be checked immediately by a Gram-stained smear and pH and, in 24 hr, as growth with marked pH change on glucose peptone agar. Direct agglutination of the broth-grown suspension with specific antiserum usefully checks strain identity and may indicate associated contamination by failure to produce complete clearing of the suspension.

Inclusion of simple plant tests for a suitable range of dilutions (10^7 - 10^{10}) at this stage provides a regular check on maintained symbiotic capacity.

The manufacturer will need to be able to evaluate the bacteriological and quantitative status of his broth cultures, by the use of some if not all of those tests, if he wishes to avoid waste of time and materials producing cultures fated for rejection. At the very least he should be checking total growth turbidimetrically, determining broth pH and examining Gram-stained smears for any morphological or staining abnormality.

The role of the control laboratory at this stage will depend on local circumstances and viewpoint. When an industry is small and still gaining experience there is a strong case of close liason between the control laboratory and the manufacturer, even when the latter might, on the face of it, possess fair bacteriological expertise.

Later, as more experience is gained, the manufacturer should be able to accept routinely responsibility for ensuring that the broth will be good enough to produce a satisfactory peat culture. It could still be possible that the control laboratory would retain the right to utilise a fuller array of tests as an overall check on a manufacturer's competence.

Tests with Manufactured Peat

This is the most important check point in determining the quality of legume inoculants. Freshly made inoculants with non-sterile peat should still have sufficient rhizobial dominance to permit valid plant counts. It will not however necessarily detect substitution by, or mixture with, another rhizobium nor will it serve as a check on any variation in symbiotic capacity. There can therefore still be a place for a simplified, but quantitative, plant infection count; strain authenticity can be checked by culturing from colonies in the plate count and applying a simple serological test.

Cultures based on sterile peat carrier will be readily counted by the plating method; in that case any significant contamination will justify culture rejection.

Tests of Purchased Peat Cultures

There is certainly a place for cultures being checked at their retail outlets to determine the quality of the produce immediately available to the farmer and whether there are difficulties in the handling and storage of cultures after manufacture. This can be most important in the earlier stage of developing an inoculant industry.

The plate count should still be quite reliable for inoculants based on sterilised carrier and even, according to the set standard, for reasonably good non-sterile peats. It may be necessary however to resort to a plant infection count as well.

Records and Certification

It is imperative, in the interests of all parties, that records concerning each manufactured batch should be complete, accurate and properly organised. This would include information as to recommended legume(s), strain(s) used, date of manufacture, batch number, expiry date and the results of tests at: (1) the factory; (2) the control laboratory.

The package itself should state clearly and unequivocally, the host(s) for which it is recommended, the batch number, the expiry date and a certification authorised by the control laboratory.

Finally one should comment as to the way in which the control of legume inoculants may be given adequate muscle. My own experience

leads me to favor a system of education and voluntary cooperation in an inoculant certification scheme backed up by a complete testing programme. Whilst it is difficult, if not impossible, to define microbiological standards in legal terms, it should be easy for the use of a certificate to carry with it all necessary safeguards. Then it would be necessary for the consumer and his advisors to look for such certification in the purchased cultures. This has worked well in Australia.

CONCLUSIONS AND RECOMMENDATIONS

It is important to ensure that inoculants contain sufficient of the right kind of rhizobia to cause fully effective nodulation of legumes for which they are recommended, under a range of practical circumstances. The history of legume inoculation shows many cases of failure to ensure adequate quality in commercial inoculants. Australian experience provide a basis for consideration as to how control may be effected and why it is a continuing necessity. Suitable standards could be 10^8/g peat culture as a suitable minimum for freshly manufactured peat, 10^7/g for peat at the retail level.

A control programme is recommended to take care of the selection, recording, maintenance and use of approved rhizobial strains. Tests of broth culture are described which enable the number of rhizobia to be assessed, the presence of contaminants to be detected and the authenticity of a rhizobial culture to be determined. The main control on culture quality can most appropriately be applied to the manufactured peat, either by means of a plate count or a combined plate and plant infection count; the relative usefulness of the two being dependent on whether the culture is based on sterile or non-sterile carrier. However similar tests with cultures purchased at normal retail outlets remain an important link between manufacturer and consumer and can pick up factors responsible for any determination at that stage. A sound system of records and certification needs to be developed and linked with essential information on the package so as to enable all cultures to be tracked back to their origin, and to show the consumer whether or not the inoculant has been approved by the independent testing authority. It is considered that more can be achieved by cooperation and liason, coupled with a well publicised system of certification, than by engaging in the complexity of legislation, except so far as the latter might provide a general legal cover for the control system.

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GROUP DISCUSSIONS

NOTE: Group discussions, held on four afternoons of the Workshop, are summarized below. These accounts do not represent verbatim reports; rather, they are a synthesis of participants' views compiled from notes and tape recordings available to the editors.

Session 1: More Effective Use of Legumes in the Tropics

1.1 Grain legumes in polycrop systems. (Chairman: Dr. James Moomaw, Asian Vegetable Research and Development Center, Taiwan.)

Polycrop systems include both simultaneous and sequential mixed cropping and imply a more efficient use of resources within the farmer's socio-economic circumstances--to maximize yield with minimum risk, minimum input and maximum ecological stability. These advantages benefit the small farmer, but impose a more complex management structure on his operations.

The crop components need to be identified and compatible crops determined so that the legume contribution can be maximized within the limits set by the considerations above, and thereby realize its particular potential for nitrogen utilization and contribution to soil fertility.

To achieve maximal yield and N_2-fixation, limiting factors due to environment, disease or pest, limitation due to features of the legume: *Rhizobium* component and to managerial practices need to be recognized and controlled, according to particular circumstances and ease of manipulation.

A general investigational model might be formulated to help localized choice of strategies. The following seem likely to require attention:

(i) Causes of unresponsiveness in the legume: whether due to unsuitability of host, inadequate nodule formation or inefficient nodule function.

(ii) Selection and breeding of legumes more suited to the polycrop system including attention to such factors as shade tolerance, water requirements, form of growth and development. The potential of hitherto little used legumes needs to be considered.

(iii) Need for seed inoculation, availability and proper use of fully effective specific rhizobia; environmental factors affecting their use.

(iv) The fertility of tropical soils; soil factors specifically limiting the nodulated system.

(v) Management of the polycrop: spacing, planting times, cropping sequences, appropriate levels of technology.

1.2 Maximizing the use of legumes as animal fodder. (Chairman: Dr. A. Sheldon Whitney, NifTAL Project, University of Hawaii.)

In practice the pasture legume is used as an important contributor to the nutrition of the grazing animal (whether a producer of milk, meat, hide or fibre, or used for draught purposes). In this way use is made of large areas of non-arable, often less fertile, land although the production of animal forage can involve more fertile areas. The establishment and management of legumes for this purpose involves the host plant in relation to other pasture species, its association with rhizobia and their joint interaction with soil and other environmental factors and with the grazing animal.

1.2.1. Plant factors.

Pasture legumes are available for a wide range of conditions in the humid tropics but more need to be sought and selected to provide a stable source of higher protein for the target animals in a particular ecological zone. Whether the legume is to be grazed or harvested for forage (or used at different times for both) will influence criteria for selection. A sufficiently wide germ plasm collection is needed.

In considering the suitability of a legume as a source of animal fodder in the humid tropics it will be necessary to extensively evaluate: (a) its suitability as animal feed; (b) its ability to establish (with good initial growth, early and effective N_2-fixation); (c) its nutritive requirements; (d) its ability to grow into the dry season; (e) to persist in spite of adverse environmental conditions, disease and pest attack; and (f) its demands on management. The semi-arid tropics will share the same requirements with additional need for tolerance to water stress and high salt concentration.

Other useful characters include relative ability to do well on poor soils, (including acid soils and those low in phosphorus) and bush growth habit. Response relationship to soil treatment, tolerances and requirements will need to be defined; adaptation and low input requirements will be the goal.

1.2.2 The *Rhizobium*

Early effective nodulation is the key to successful establishment of legumes amongst other pasture components. Therefore, unless the soil is already well populated with rhizobia able to achieve such nodulation, attention must be paid to the provision and use of rhizobial inoculant which will be:

(i) fully effective with the chosen legume.

(ii) provided in sufficient number and of a kind as will establish itself in the soil, give early nodulation, and compete successfully with any less effective rhizobia already present.

(iii) as tolerant as possible of conditions encountered in the soil.

Related short-term investigations should include:

(i) tests to establish at the local level whether inoculation is needed.

(ii) simpler screening techniques, to enable the plant breeder, agronomist and the like to test legume-*Rhizobium*-soil combinations.

(iii) simpler techniques for correctly assessing N_2-fixation under field conditions.

In the longer term there needs to be:

(i) fine tuning between rhizobial strains and legume varieties.

(ii) development of inoculant delivery systems that will enable the rhizobia to withstand the more severe conditions likely to be encountered in the tropics.

Training in the handling of rhizobia is required together with more familiarity with and use of the IBP Manual No. 15 (J.M. Vincent--A Manual for the Practical Study of the Root-nodule Bacteria. Blackwell Scientific, Oxford, 1970). This manual is available in a Spanish edition and a French translation is projected. The need for easier availability of the English version was noted.

More communication amongst workers is urgently required.

Standards--Adequate and relatively uniform quality standards and control procedures need to be applied.

Role of developed countries and international centers--These can help with expertise and provision of tested rhizobial strains; developing countries can undertake isolation and selection; international centers can span the two viz--by local screening and establishing rhizobial (as well as plant) germ plasm collections. (The availability of cultures from ICRISAT and CIAT, the NifTAL Project and progress towards incorporation of data on rhizobial strains in the World Culture Collection Records were noted.)

1.2.3 Soil.

It is necessary to determine suitability of land for pasture legumes noting that economic, climatic and social, as well as soil, circumstances are likely to determine whether land is used for cash crops, harvested fodder or grazing by animals. Commonly the last will be restricted to less fertile land whose relatively uncontrolled variables may (but need not necessarily) set a relatively low ceiling on its productivity. The legumes used for this purpose will therefore need to be adapted to certain consequent stresses. On the other hand ceiling of productivity of forage production, at least in favoured temperate situations, need not be low and under these circumstances re-cycling of nutrients

may permit high inputs. However, this is hard to match in tropical regions where large inputs are generally not feasible, and economic use of legumes for the grazing animal will depend on such factors as the following:

(i) availability of suitable plants, providing forage with good nutrient quality and free of toxic effects.

(ii) rhizobia adapted to chosen legumes and best able to form an effective nodule system under practical conditions of environmental stress.

(iii) availability of cheap and easy techniques for establishment.

(iv) ability of the pasture legumes to provide relatively high yield of animal products with relatively low inputs, preferably with a high proportion of re-cycled nutrients.

(v) availability of animals capable of responding to the established legume component and yielding products directly valuable to the farmer and, in some circumstances, marketable on a sound economic basis.

1.2.4 Animal-plant interactions.

Even a small amount of a legume component has been found to cause a considerable improvement in animal production. Legumes such as *Stylosanthes humilis* are presently available to improve large areas with suitable rainfall.

Problems include social aspects of the use of animals: number v quality; the implications of uncontrolled communal grazing; difficulty in obtaining seed of varieties known to be suitable or getting fertilizer to where it is needed. Toxic effects of some legumes also constitute a barrier to more widespread use.

The better use of legumes as animal forage requires:

(i) ability to establish the desired legume with minimal inputs and so as to set seed generally in the presence of the grazing animal and in competition with other pasture components. The establishment of an effective N_2-fixing nodulation by means of natural or introduced rhizobia will be an integral part of such a process.

(ii) availability of animals able to make appropriate economic use of the pasture (as noted above).

(iii) management of grazing practices (e.g. intensity, animals used) to secure maximum animal production compatible with the needs of the pasture for proper maintenance and balance. This will involve knowledge of the structure of the legume component, its grazing tolerance and the possibilities of mixed grazing.

(iv) knowledge of causes of plant toxicity and how this may be avoided, or the risks reduced, by breeding plants with toxin absent or at a low level, and/or management of grazing program to avoid poisoning.

1.2.5 General.

In conclusion, it was pointed out how the answers to these

questions were largely concerned with:

Adaptation: including response to inputs and the accumulation of data to permit prediction.

Establishment and persistence: both plant and *Rhizobium*, often under stress conditions.

Better understanding of these could be expected to reduce failures in attempts to utilize legumes as source of animal feed.

Session 2: Matching the Symbionts to the Environment.

2.1 Breeding and management of legumes. (Chairman: Dr. James L. Brewbaker, University of Hawaii.)

Preliminary consideration was given to such possible objectives as:

(i) tolerance to low available phosphorus and soil acidity.
(ii) increased root and/or nodule volume.
(iii) better compatibility with rhizobia and mycorrhiza.
(iv) adaptation to polycropping and general low input systems.
(v) increased photosynthetic efficiency, reduced photo-respiration.
(vi) quality and yield of plant product (Should yield have precedence?).

Progress seems to require:

(i) sufficient genetic variability and the opportunity for it to be expressed and evaluated against a relatively non variable environment.
(ii) large plant populations.
(iii) simple techniques to identify genetic variability.

Points from the group discussion can be summarized:

2.1.1 Breeding objectives.

It was noted that a breeding program may evolve from a general understanding of principles to one designed more specifically to meet more local site, environmental and managerial requirements. On the other hand there is a need to define the intended clientele and their capability of providing inputs to achieve this objective and for setting up clearly defined goals such as: yield, quality (including freedom from toxic constituents), phosphorus uptake (possibly involving compatibility with mycorrhizae), resistance to disease and pests (or characteristics permitting avoidance of damage), tolerance of heat and water stress, improved root system, symbiotic tolerance to relatively high levels of soil-N and low input.

It will be the breeder's responsibility to assess features of legume use and the relative importance of stresses, and to examine the material at his disposal in relation to locality and people. A multiplicity of small restrains may result in a relatively large deficiency. On the other hand, breeding for specific

adaptations may be too time consuming so that breeding to some total environments may be better. Some stresses will be widely distributed; others might cluster in groups. Stress tolerance may entail loss of yield or decrease in quality or both. This needs to be determined and any opportunity to separate them utilized.

The genetic basis and needs of the symbiotic system require close attention early in any program but there was some difference of opinion as to the stage at which this should be taken into account. The early program might utilize combined-N to assure retention of potentially useful lines; alternatively the plants might be tested and selected against one or several chosen symbionts to avoid the risk of building in difficult levels of incompatibility. The question as to the best way to reconcile the large-scale procedures of the plant breeder with present time-consuming methods of evaluating symbiotic capacity was thought to be one for International Centers and those in Developed Countries.

2.1.2 Methods and techniques

Resort to widely based germ plasm and screening under conditions of ultimate use was generally advocated. An approach would be to obtain increase by selection until a production plateau was reached and then breed to exceed such a level when parameters had been more clearly defined. New techniques (such as haploid pollen and increased recombination potential by the outcrossing of male sterile material) may be developed for legumes.

2.1.3 The role of the plant breeder needs to be defined for developing countries, where he will be expected to deal with local short-term, as well as the more traditional long-term objectives. International centers could be expected to play a major role in utilizing plant breeding expertise and germ plasm stocks close to the developing scene. There might be a place for primitive legumes from developing countries to provide a wider genetic base.

A team approach is likely to be available at such centers, but training or personnel adaptation will often be needed. The breeder will need to ask the right genetic questions relative to the legumes likely to be of use at the locality level. The problems of the breeder operating with little technical back-up need to be considered, along with the kind of training he requires for this kind of assignment. The question was asked: Is the present training suitable and realistic?

2.2 Selection of rhizobia. (Chairman: Dr. Martin Alexander, Cornell University, Ithaca, New York, U.S.A.)

Groups addressed themselves to a range of questions posed by the Chairman. Their views can be summarized:

2.2.1 Desirable traits:

(i) Ability to nodulate early and fix N_2 at a high level with crops of proven or potential value, when proven necessary under stressed conditions. When possible, attention should be given to both local needs and those of other regions.

(ii) Ecological competence, both free-living and when associated with the host including: (a) survival in the inoculum, on the seed, on the root and in the soil; (b) competitiveness; (c) resistance to toxic factors (natural or man-made) and antagonists; (d) general performance under field conditions including specific adaptation to soil and climates; (e) the ability to fix N_2 in the presence of soil or fertilizer - N. There needs to be some concern with the form in which the inoculum is presented to the plant and the possibility of new inoculation techniques.

(iii) Suitability for large scale inoculant production.

2.2.2 Source, selection and storage of strains

It was generally agreed that at present, and in the immediate future, there would have to be dependence on natural rather than "laboratory-engineered" strains. The latter may prove advantageous in combatting man-made stresses (e.g. pesticides, where there may have been little opportunity for selection), and so may become increasingly important in the future.

Selecting and testing of strains need to include work and decisions at the local level but free exchange of strains (without bureaucratic restraints) and of information as to their performance (preferably in comparison with standard strains) was stressed with emphasis on improved storage methods and documentation (including data on effectiveness and tolerance under specified conditions). The general use of lyophilization was recommended.

A need was recognized for more centralized rhizobial germ plasm collections at least duplicated at several developed and international centers. Authenticity of held cultures needs to be rigorously established. A central computer record of cultures held at the various local levels was commended for speedy implementation. The current status of recording for the World Federation of Culture Collections was noted.

2.2.3 Methods.

It was agreed that the IBP Manual No. 15 should be revised and/or reprinted.

Although no rapid test, independent of the plant, is presently available for general use, such would obviously be most advantageous if sufficiently correlated with symbiotic performance (as e.g. seed isoenzyme patterns of *Stylosanthes*). Any such predictions would need to be verified under field conditions.

2.2.4 Personnel.

The present shortage of trained personnel was noted and emphasis placed on the need of training in the more practical aspects of rhizobiology, in developed as well as in developing countries. Training at a local level is also important.

Movement between countries for this purpose needs to be facilitated, but training by different agencies needs to be coordinated and participants carefully selected for active involvement with the rhizobia.

2.2.5 Communication.

Existing lines of communication amongst rhizobial workers need to be strengthened as does exchange of information and ideas with agronomists, plant breeders, plant physiologists, etc. Centers in developed countries and the international centers could help in this regard and extend their assistance to and beyond the local region.

The value of *Rhizobium* Newsletter as a well established means of communication was emphasized together with a need to secure its financial future--possibly to an extent which will permit wider distribution in developing countries.

2.2.6 Roles of developed and developing countries

The latter should recognize and initiate the need for help from developed countries; exchange of trained persons should be for a sufficiently long period to permit thorough familiarization with the problem. International centers play a useful role in facilitating contacts between workers.

Session 3: Criteria for Rhizobial Selection.

3.1 Legume x *Rhizobium* genotype specificity. Chairman: Dr. J.E. Burton, The Nitragin Company, Milwaukee, Wisconsin, U.S.A.)

The group was asked to consider these questions:

(i) Should plant genotypes be screened for compatibility with rhizobia? If so, when, how, in respect to what criteria (early or late nodulation, acetylene reduction) and against what rhizobia (natural, implanted; single or multiple strains)?

(ii) Should host plant cultivars be screened for N_2-fixation against a background of minimal nitrogen? Should tolerance of a higher level of combined N be sought?

(iii) Can nodule activity be extended by growth regulators or by foliar feeding?

(iv) Has sufficient attention been given to host plant morphology as for example provision of a canopy such as will maximize supply of photosynthate? Has harvesting convenience over-shadowed N_2-fixing ability?

Most of the discussion dealt with the first two groups of questions, largely the questions as to whether early maintenance of genotypes should use combined-N, leaving the latter selection to operate with a symbiotic-N background, or whether a chosen strain, or strains, should provide the initial, as well as the later background, in order to reduce the risk of perpetrating poor symbiotic capacity.

Further questions concerned selection for hosts having a wide range of symbiotic capacity, the possibility that late nodulating characteristics may prove advantageous, if it means that young fully functioning nodules are thus more available to the plant at the time of maximum N demand, and selection for tolerance of various stress factors.

3.2 *Rhizobium* colonization, persistence and inter-strain competition. (Chairman: Dr. R.A. Date, C.S.I.R.O., Division of Tropical Crops and Pastures, Cunningham Laboratory, St. Lucia, Queensland 4067, Australia.)

This discussion was mainly concerned with questions; there were few precise answers. Many of the questions were concerned with the longer term persistence of introduced strains: the common (though not invariable) difficulty in recovering them in subsequent years, the longer-term credentials of antigenic properties as basis for strain recognition, the possibility of genetic change in strains (by mutation or recombination), whether bacteriophage or the phenomenon of lysogeny plays a role. One point of view was that fluctuating circumstances in the soil from year to year are not likely to be catered for by a single strain, or even several strains, so that the widely observed condition of strain diversity was the likely outcome of the long-term operation of such variable conditions.

It was apparent that we lack adequate understanding of those ecological aspects which determine rhizobial representation in the soil and in association with the leguminous plant. Such an understanding requires techniques which are only beginning to be developed and exploited.

Session 4: Directions for Future Work.

4.1 Priorities for legume/*Rhizobium* research. (Chairman: Dr. A.S. Whitney, NifTAL Project, University of Hawaii.)

The following aspects of methodology, training needs and research were suggested by the participants as worthy of consideration:

A. Methodologies

1. Screening procedures
   -- *Rhizobium* x host and correlated factors.

-- Performance under stress: acid soils, high temperature, dry conditions, alkaline and/or saline soils, antagonism by other soil microorganisms.
-- Simple techniques for C_2H_4 assay, and for isolation of rhizobia.

2. Measurement of N_2-fixation with progressive and integrated evaluation of plant response throughout the growth cycle.
3. Techniques for the study of *Rhizobium* in soil, on roots and in nodules.
4. Inoculation methods.
5. Assessment of inoculation requirement and diagnosis of a *Rhizobium* problem.
6. Assessment of contribution of symbiotic N_2-fixation to soil-N pool and other crops.
7. Assessment and ranking of stresses (nutritional, physiological-hormonal, climatic). What are the diagnostic criteria?
8. Better standardized technique for field testing, plot size, replications, designs.
9. Laboratory-field correlations.
10. A method for obtaining *Rhizobium*-free soils.

B. Training

1. Professional training:
   a) in soil microbiology
   b) in agronomy
2. Technologist - microbiologist with agronomic experience (crop-field studies).
3. Identification and management of soil limiting factors.
4. Agronomist - to appreciate N-symbiotic factors; to define problems.
5. Inoculant production--small and large scale.
6. Quality control of inocula--basic rhizobial microbiology (purity check, serology effectivity tests).
7. Inoculation technology--how to inoculate seeds.

C. Research

1. Meaning of nodule numbers, weight (mass) and efficiency.
2. Early and late nodulation:
   a) span of active N-fixation
   b) relative to physiology of the plant (annual vs. perennial)
3. Mono vs. multi-line inoculation--to nodulate several species.
4. Frequency of inoculation in rotations.
5. Effect of N on symbiosis with particular reference to *Rhizobium* strains capable of fixing N_2 in the presence of high amount of combined-N.
6. Inclusion of bacteriocinogenic and antibiotic producing, and resistant strains.
7. Pesticide - compatible strains.

8. Plants with more efficient photosynthesis and less photorespiration.
9. Long-term ecology of *Rhizobium* in soil.
10. Better survival of *Rhizobium* on seed coats (including use of pellets and trace nutrients)
11. Symbiosis in perennial legumes (season, age, management).
12. Selection of rhizobia and plants for stress environments.
13. Survey of trace element deficiencies.
14. *Rhizobium* mobility.
15. Basic research on rhizobia, including genetics and physiology.
16. Effect of plant injury and grazing on symbiosis.
17. Allelopathy: Ways of minimizing inter-plant toxicities.
18. Breeding/selection of legumes for low light intensity conditions, as under trees or tall intercrops.
19. Mycorrhiza x *Rhizobium* interaction.

4.2 A Tropical Legume Research Strategy. (Chairman: Dr. D.L. Plucknett, University of Hawaii.)

4.2.1 Goals.

Shorter term, urgent (perhaps 5-year) and longer term (10-year) goals need to be determined for less developed countries, as well as developed countries and international centers. These determinations involve recognition of needs, a commitment to meeting these needs, plans, positive decisions and the allocation of resources.

4.2.2 Problem identification ("A researchable project is not necessarily a problem") Poor problem identification in developing countries reflects similar inadequacy in the developed. It often arises from similar failure to recognize the practical requirements of the situation, or reflects preference for a technically fashionable or more glamorous exercise in research.

Who does the identification? Certainly these should include someone familiar with the local scene, perhaps assisted by visiting consultants sufficiently aware of the local scene and requirements.

More specifically problem identification relating to legume usage could be expected to begin determination of the importance and potential of legumes to the economics of land use. Secondly legumes likely to have agronomic significance in locally adopted farming systems would need to be identified. The possibilities for neglected legumes and unusual farming systems should not be overlooked. Demonstration of their importance and investigation of rhizobial and other factors limiting production could then follow using relatively simple experiments. Without overlooking local opportunities and resources some good public relations and capitalization on results should be used to build up an awareness

of the potential of the *Rhizobium* symbiosis at all levels--from farmer to government.

4.2.3 Problem solving. (Who does the work?)

A desirable minimum team could be taken to include:

an agronomist (integrator) having an appreciation of the rhizobial side of the symbiosis.

a soil fertility expert, again with a rhizobial slant.

a field-oriented rhizobiologist, with an interest in practical problems, willing and able to cooperate with non-rhizobial members of the team.

At the same time, it was recognized that such a team might not be realizable. Problems might then have to be tackled by one or two workers who, although only partly familiar with the rhizobial side, would be sufficiently experienced and aware of its importance to give it adequate emphasis.

Interchange of information, and even linked experiments between countries and centers, could provide mutual support and stimulate new work. Attention was drawn to *Rhizobium* Newsletter (ed. J.M. Vincent, University of Sydney, Australia) as a well established means of information exchange.

4.2.4 Training.

It is apparent that the present training of agronomists, other agriculturists and rhizobiologists, even in developed countries, is inadequate as preparation for identifying and solving problems concerned with the nodulated legume.

There is a need to train local people, preferably within the same region or in a not too unrelated environment.

Questions for consideration are:

(i) Do the right people attend the right courses or are decisions too often based on administrative seniority?

(ii) Should there be special courses to condition administrators to an appreciation of the legume-rhizobial system?

(iii) How do we best use a returning trainee? How to separate him from cherished methods and equipment? How do we get him to appreciate that nitrogen accumulation (and/or yield) is the name of the game, and that rhizobial genetics, though fascinating, does not seem likely to solve urgent practical problems of tropical legumes?

CONCLUSION

In concluding the Workshop, Dr. A.S. Whitney briefly referred to the value of the contributions to the NifTAL Project by the participants. The NifTAL project will endeavour to support legume programs by: (a) providing training courses (the first of which

was to follow the Workshop); (b) serving as a germ plasm store for rhizobial strains (particularly those likely to be of use in tropical regions) and (c) investigating such areas of the legume--*Rhizobium* association as are favoured by its location in Hawaii.

Those attending the Workshop were invited to collaborate with NifTAL, particularly by supplying well documented and potentially useful strains for the collection.

Dr. A.M. Pinchinat expressed on behalf of the participants appreciation of hospitality extended to them by NifTAL during the course of the Workshop.