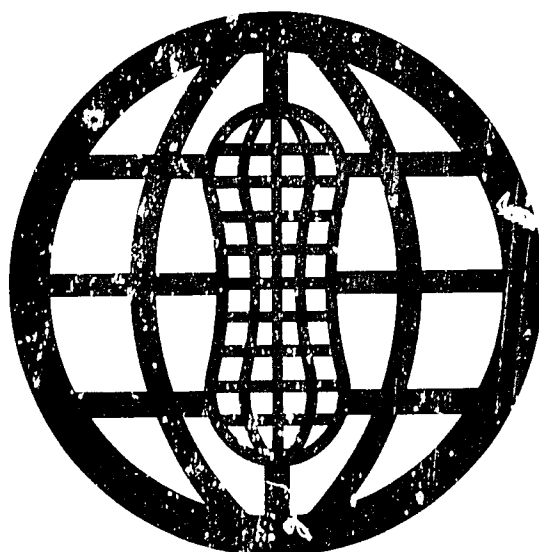


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**1987/88**  
**Annual Report of the**  
**Peanut Collaborative Research**  
**Support Program**  
**(CRSP)**



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## Foreword

The Peanut CRSP has completed six full years. The program is maturing with many useful results being obtained, and in several cases the technology is being transferred to the end user. Staff training is now resulting in improved research programs in host countries as trainees are back on the job in CRSP related activities.

We are looking forward to a Triennial Review during the seventh year of the program and anticipate another three-year extension.

I have reassumed the role of Program Director after a profitable two-year Joint Career Corps assignment with the USAID Mission to the Philippines. Thanks go to Dr. Tommy Nakayama for a job well done during my absence. Appreciation is extended to all who have contributed in making the program successful: Administrative Secretary; Accounting Assistant; University of Georgia Agricultural Business Manager, International Development Director, and Agricultural Communications Staff; Board of Directors; Technical Committee; USAID Program Manager; BIFAD Representative; External Evaluation Panel; and U.S. and Host Country Principal Investigators, Cooperators, and support staff. Thanks also go to the University of Georgia Administration for their support of the CRSP. You make the program a success and the work a pleasure.



David G. Cummins  
Program Director  
October 1988

# Table of Contents

EXECUTIVE SUMMARY .....	iii
INTRODUCTION .....	1
Features of Peanut CRSP .....	1
Goal .....	1
Objectives .....	1
Specific Objectives and Strategy .....	2
Networks .....	3
MANAGEMENT ORGANIZATION AND ACCOMPLISHMENTS .....	5
Management Entity .....	5
Board of Directors .....	6
Technical Committee .....	7
External Evaluation Panel .....	8
Coordination with AID and BIFAD .....	8
CRSP-ICRISAT Coordination .....	8
Training .....	9
PROGRAM SUPPORT .....	9
Summary, Table 1 .....	10
Allocation and Expenditure of Program Funds, Table 2 .....	11
Cumulative Budgets and Expended Program Funds, Table 3 .....	12
Management Entity Costs, Table 4 .....	13
ANNUAL PROGRESS REPORTS FOR PROJECTS .....	14
Global Nature of Peanut CRSP .....	14
Introduction .....	15
TX/BCP/S,BF,N -Disease-Resistant Peanut Varieties for Semi-Arid Environments .....	16
NCS/BCP/TP - Peanut Varietal Improvement for Thailand and the Philippines .....	40
TX/MM/S - Mycotoxin Management in Peanut by Prevention of Contamination and Monitoring .....	63
GA/IM/BF - IPM Strategies for Peanut Insects in SAT Africa .....	86
NCS/IM/TP - Management of Arthropods on Peanuts in Southeast Asia .....	106
GA/PV/N,TP - Peanut Viruses: Etiology, Epidemiology, and Nature of Resistance .....	135
NCS/SM/TP - Influence of Soil Microbiology on Nitrogen Fixation and Growth of Peanuts in Thailand and Philippines: A. Rhizobia .....	150
TX/SM/TP - Influence of Soil Microbiology on Nitrogen Fixation and Growth of Peanuts in Thailand and Philippines: B. Mycorrhiza .....	170
AAM/FT/CAR - Peanut Utilization in Food Systems in Developing Countries .....	188
GA/FT/TP - Appropriate Technology for Storage/Utilization of Peanut .....	221
AAM/FT/S,BF - An Interdisciplinary Approach to Optimum Food Utility of the Peanut in SAT Africa .....	240
GA/PH/CAR - Postharvest Handling Systems for the Small Peanut Producer .....	293

## Executive Summary

The Collaborative Research Support Programs (CRSP) are a product of a program funded by the U.S. Congress to address food needs and provide developmental assistance in research to developing countries around the world. The CRSP's were developed by the U.S. Agency for International Development and participating U.S. universities coordinated through the Board for International Food and Agricultural Development. The programs are implemented and conducted collaboratively by the U.S. universities and host institutions in the developing countries.

The Peanut CRSP (one of seven active CRSP's) is characterized by the following specific features:

- Targeted effort based on constraints identified during the planning process and modified as needed on a continuing basis.
- Efficient design of four U.S. universities and a structure to maximize program expenditures and impact and minimize management costs.
- Global impact encompassing three regions (Semiarid Tropical Africa, Southeast Asia, and the Caribbean) and including nine host countries (Senegal, Burkina Faso, Niger, Nigeria, Mali, Sudan, Thailand, Philippines, and the English speaking Caribbean countries).

### Accomplishments

A summary of accomplishments made during the year in research, training, and program management follows.

*Research*—The CRSP was designed around major constraints to production and utilization, each addressing specific needs in developing countries with regional or global implications.

#### Constraint: Low yielding cultivars

- Two peanut cultivars were officially released in Thailand. Khon Kaen 60-1 yields more and has larger seed than the commonly grown cultivar, and Khon Kaen 60-2 has improved boiling characteristics.
- UPL Pn-6, locally known as Biyaya 6, was released in the Philippines. The new cultivar out yields the local cultivars and has moderate resistance to leafspot and rust.
- NC 10c was released in North Carolina because of its resistance to *Cylindrocladium* black rot, a serious disease in the area.
- A drought tolerant line yielding 20% over present cultivars has been developed in Texas and Senegal and should be released to growers in the near future.
- A leafspot resistant line is nearing release. The line developed in Texas and Senegal yields 17% higher with 25% lower leafspot incidence than present cultivars.

#### Constraint: Mycotoxin hazards to health.

- Screening peanut cultivars for resistance to soil-borne plant pathogens under field conditions has revealed that some select breeding lines have moderate levels of resistance to invasion by the aflatoxin producing fungus, *Aspergillus flavus*. Little is known of the mode of resistance, but recent research points to the presence of a protein synthesized shortly after fungal inoculation, termed "pathogenesis related protein," that contributes to the defense.
- The aflatoxin content of contaminated peanut paste (butter) was reduced by approximately 80% of the original concentration after boiling for about two hours in water.

—Research continued to transfer to the village level in Senegal the use of highly adsorptive clays for removal of aflatoxin from crude peanut oil, and adaptive studies showed over 90% removal. Subsequent poultry feeding trials in Texas have shown that a small percentage of the clay in an aflatoxin contaminated ration prevented accumulation of aflatoxin in the poultry livers.

Constraint: Pest damage to crops.

—Coordinated research in Philippines, Thailand, and North Carolina has identified peanut lines with multi-resistance to several insects damaging peanut at an economic level. Resistance to the major insects was observed at each location. Subsequent development of cultivars with resistance or tolerance to insects will contribute to low-input pest management packages for sustainable agriculture programs.

—Thrips are common insects on peanut in North Carolina, Philippines and Thailand. Infestation comes early in North Carolina with economic damage, but appears later in Philippines and Thailand with often subeconomic damage. Integrated pest management procedures should take this difference into consideration. Tolerant varieties should contribute much to maintaining damage at subeconomic levels in Philippines and Thailand.

—Delay of harvest beyond maturity significantly increased termite damage to the unharvested pods in Burkina Faso studies. Cultivars were identified in companion tests that had resistance to termite damage even at the late harvest dates.

—Peanut lines tolerant to foliar feeding insects have been identified in Georgia studies, and will be evaluated in Burkina Faso. These lines will be incorporated into cultivars to contribute to the development of sustainable, low-input production systems.

—Many growers have reduced or eliminated peanut production in Northern Nigeria because of the rosette epidemics of 1975 and 1985. CRSP research has helped define the etiology, epidemiology, and nature of host-plant resistance to rosette. Resistant cultivars are available for the longer growing seasons of Southern Nigeria but not for the shorter seasons further north. Three lines with shorter maturity and rosette resistance have been identified and recommended for release that will contribute to the solution of the problem. A breeder being trained under the CRSP will continue efforts to introduce resistance into short season cultivars adaptable to Northern Nigeria.

—Preliminary studies on Peanut Stripe virus (PStV) in Thailand indicate that yield reductions due to PStV may be higher than those observed in the United States. Symptom expression in Southeast Asia is also more severe than in the U.S. Control measures are under development to reduce yield losses from PStV.

Constraint: Biological barriers—soil microbes.

—Earlier studies in Cameroon supported by the CRSP, showed a 28% increase in yields of a local cultivar 28-206 when inoculated with a particular rhizobium species, NC92. A Cameroonian company was preparing to produce the rhizobium for local use.

—More consistent yield responses to rhizobium inoculation of peanut was obtained in Thailand when molybdenum was applied with the inoculum.

—Evidence has been accumulated that documents the beneficial effects of mycorrhizal fungi on peanut growth. Differences in efficiencies of various species have been demonstrated. Mycorrhizal colonization in the roots increases yields, phosphorus and manganese uptake, rhizobium infection/nitrogen fixation, and water uptake, and decreases nematode damage.

Constraint: Food source—supply and quality

—Spreadable pastes containing more than 90% peanuts have been developed for use on bread and crackers. These spreads are processed from raw, blanched peanut kernels and are stable at room

temperature or under refrigeration, depending upon processing schemes and ingredient formulation. Most promising flavors include Cheddar cheese, chocolate and tangerine. Evaluation of spreads in the U.S. and Philippines indicate high acceptability. These products are high in protein, and the favorable protein:calorie balance is especially valuable to consumers in developing countries.

- A peanut lipid film tempered with glycerol has been developed from aqueous extracts of peanut. This film has unique properties of elasticity, strength and permeability to water and air. It is envisioned that the film will have application as an edible coating, skin or package for a wide range of food and non-food materials.
- Sensory qualities of ground chicken extended with defatted peanut flours reduced cooking shrinkage, increased tenderness, and were acceptable to Thai tastes. Peanut use in such products could increase the amount of protein-rich food available to consumers.
- The length of time peanut can be stored without refrigeration is important for maintaining constant food supplies in developing countries. Steam or hot water blanching and storage in jars allowed for room-temperature storage up to three months without the development of rancidity.
- “Mish” is a yogurt-like product made from milk in Africa. An acceptable “mish” was produced from peanut milk that was similar in texture, consistency, and flavor to traditional “mish” prepared from milk.
- Premature harvest of peanut has resulted in poor quality peanut butter production in Jamaica. Physical characteristics and sensory evaluations have been determined on peanut at different stages of maturity and are providing the basis for recommendations on how to determine optimum harvest dates. Greater acceptability of local peanut by processors for peanut butter production should result.
- The Caribbean countries can probably maintain a sustained production of peanut to meet consumer needs with the use of a suitable cultivar. However, current methods of harvesting, threshing, and shelling result in high cost and low quality peanut. Farm surveys indicate about 20% of production costs are associated with threshing, currently a hand operation. Research recently initiated to develop appropriate postharvest handling systems for the small peanut producer is concentrating on development of efficient mechanical threshers that can be constructed from locally available, surplus materials.

*Training*—Improvement of the research capability of various key individuals in the host countries and the U.S. is an integral part of the CRSP.

- 13 host country scientists visited collaborators at U.S. institutions for laboratory and field training in research methodologies, reviewing accomplishments, and planning future activities.
- 10 host country scientists visited “third-country” scientists or institutions for short term training or workshops.
- 25 host country students supported by host country CRSP budgets were enrolled in programs leading to graduate degrees.
- 24 students (22 U.S., 2 foreign) were provided full or partial support from the U.S. CRSP budgets in programs leading to graduate degrees.
- 14 U.S. scientists made site visits to consult with collaborators. Total time spent was 225 days or 0.86 man years.

*Resource Management and Communication*—Emphasis in resource management was placed on efficient use of human and financial resources, maximum utilization of funds for program activities,

minimum expenditures for management, and emphasis on communication activities to encourage dissemination and utilization of results.

- Program management was provided by the Program Director, Administrative Secretary, and Accounting Assistant. Management support accounted for 17.5% of AID funds.
- Direction was provided by the five Board members, four Technical Committee members, University of Georgia Agriculture Business Manager, and Director of International Development, the USAID Project Manager, and the External Evaluation Panel. Support for these groups accounted for 8% of the Management budget.
- The Board of Directors and Technical Committee met three times, including one meeting by conference call. One External Evaluation Panel member and a special consultant were employed one time each.
- Emphasis was placed on developing networks among international groups for coordination of research and dissemination of information. Active participation now exists with at least seven groups. Special effort is made to coordinate program activities with ICRISAT, and an ICRISAT staff member is on the CRSP Board of Directors.
- The CRSP was actively involved in two international workshops.
- International Arachis Newsletter* is published twice per year cooperatively by ICRISAT and the Peanut CRSP.

## Introduction

The peanut, *Arachis hypogaea* L., is an annual legume native to South America, likely originating on the eastern foothills of the Andes in the area that is now southern Bolivia and northern Argentina. It is grown in most tropical, subtropical, and temperate countries between 40 degrees north and 40 degrees south. Estimated annual production of peanut is about 18 million metric tons on 18 million hectares. More than half of the production is in developing countries, and yields are often much lower than the world average.

Peanut is an important oil, food, and feed source worldwide. An estimated 80% of the world production is extracted for cooking oil. Uses vary worldwide. For example, India the largest producer of peanut uses essentially all the production for oil, while in some countries of Semi-arid Tropical Africa over half of the production is consumed directly as food by the subsistence farmer who produced them. Peanut is well suited to production by small farmers in developing countries, but production is low and erratic.

Research needs are great. In a USAID survey, peanut research in developing countries was rated highest priority, excluding small ruminants, sorghum and millet, and bean/cowpea, to improve the well being of the small farmer in developing countries. In implementing the Peanut Collaborative Research Support Program (CRSP), the Board for International Food and Agricultural Development (BIFAD) Joint Research Committee recognized the essential role of research to relieve constraints and realize the great potential of peanut to provide food and cash income in developing countries.

The program is funded through "Title XII-Famine Prevention and Freedom from Hunger" under the "International Development and Food Assistance Act of 1975", and the participating U.S. and host country institutions. The Peanut CRSP was implemented 1 July 1982, and including a three-year extension, continues through June 30, 1990.

### *Features of Peanut CRSP*

**Targeted effort**—Constraints were identified and targeted research objectives were established for each host country and U.S. institution. Collaborators were identified or described in the planning process.

**Efficient design**—Four U.S. universities allow for a manageable CRSP, with minimum management expenditure and maximum program expenditure. The universities are Alabama &M, Georgia, North Carolina State, and Texas A&M.

**Global impact**—Collaboration with 9 host countries provides impact into 3 major regions; Semi-arid Tropical Africa, Southeast Asia, and the Caribbean. Specific countries are: Senegal, Mali, Burkina Faso, Niger, Nigeria, Sudan, Thailand, Philippines, and the English speaking Caribbean Countries through the Caribbean Agricultural Research and Development Institute (CARDI).

### *Goal*

The goal of the CRSP program under the USAID's Agriculture, Rural Development and Nutrition program is to improve the availabilities and consumption of food, and increase the income of the poor majority, and at the same time maintain or enhance the natural resource base.

### *Objectives*

The Peanut CRSP has two general objectives common to all projects.

1. Support research programs in the U.S. and host country institutions to:

—develop cultivars, cultural and pest management practices, and utilization processes that would lower costs and stimulate peanut utilization as a food resource.

2. Improve the research capability of U.S. and host country institutions by:

—support of programs in terms of equipment, supplies, travel, and personnel.



- offering short term and degree oriented training programs for host country staff at U.S. institutions, and degree training for select U.S. students.
- providing on-site consultation in the host countries by U.S. scientists.

### *Specific Objectives and Strategy*

After six years of operation, the Peanut CRSP has evolved through several stages or phases in addressing constraints and integrating resources from USAID/Science and Technology/Agriculture, participating U.S. universities, and the collaborating host country institutions. Additionally, the program is enhanced through coordination with the in-country USAID Missions, and other international groups including ICRISAT (International Crop Research Institute for the Semiarid Tropics), ACIAR (Australian Centre for International Agricultural Research), and IDRC (International Development Research Centre-Canada).

### Planning

The CRSP was developed around constraints limiting peanut production and utilization. Socioeconomic constraints of food supplies of the general population and low income status of small farmers and processors provided impetus for the program. These constraints were addressed through solutions to biological constraints of low yielding cultivars susceptible to diseases, insects, and drought; mycotoxin contamination resulting in health hazards; yield losses due to pests; physiological and soil microbiological barriers; and inappropriate handling, storage, and processing of peanut for food. Economic situations in relation to peanut production and utilization were observed and analyzed.

### Implementation

Through mutual agreements, an integrated relationship was developed among the Management Entity, the U.S. Universities, and the Host Country Institutions based on support and coordination by USAID. Additional support and program enhancement came initially through coordination with the in-country USAID missions and with ICRISAT. An ICRISAT staff member holds membership on the CRSP Board of Directors.

### **Interrelationship between CRSP project and Host Country constraints.**

Projects	Constraints Addressed/Impacts	
	Biological	Economic
Breeding (Texas/West Africa, North Carolina/ Southeast Asia)	enhancement of genetic productivity	increased incomes
Mycotoxin (Texas/West Africa)	product quality to health	lower hazards
Pest Management (Georgia/West Africa, Southeast Asia; North Carolina/ Southeast Asia)	tolerance to stress and integrated pest management	sustainable agriculture
Soil/microbiology (Texas and North Carolina/Southeast Asia)	enhanced nitrogen fixation and nutrient and water uptake	sustainable low input agriculture
Food Technology/Post- harvest (Georgia/ Southeast Asia, Caribbean; Alabama/West Africa, Caribbean)	improved type and quality of products added incomes	better nutrition and increased value

Implementation of a research program that addresses economic concerns through alleviation of biological constraints with the multi-institutional involvement has evolved into additional concerns with environmental impacts.

An enhanced environment results from soil fertility improvement through nitrogen fixation, reduced soil erosion by the closed canopy characteristic of the crop, breeding for better water use efficiency, and a reduction in the use of pesticides through an integrated pest management approach that includes host plant resistance to diseases and insects. Bio-compatibility of system components and the environmental implications is considered in developing research initiatives.

Sustainability of the agroecosystems is of major concern within the general realm of environmental concerns. Peanut fits into a variety of cropping systems providing added produce as well as supplying nitrogen to the total system and providing ground cover. Coupled with breeding for stress resistance to be more efficient users of water and soil nutrients and for tolerance to diseases and insects to lower external inputs, the CRSP is contributing greatly to the practice of sustainable agriculture.

As a part of the total environment, the CRSP is contributing to social improvements in the way of increased incomes, better foods and nutrition, development of human resource capability, and providing a setting for more meaningful employment.

### Procedural Concepts

The CRSP has a dynamic planning process. Annual plans-of-work are developed to stay abreast with changing problems and needs and to "fine-tune" research approaches and methodologies. The annual plans are developed jointly by U.S. and host country researchers to encompass the major initiatives of both groups. The plans enable annual evaluations and assessments of progress.

An intensive review and planning process is accomplished every three years as a basis for any necessary changes in program direction and for grant renewal by USAID. Sensitivity to host country needs is a prime mover of the triennial review and planning process.

The CRSP meeting held in conjunction with the Annual Meeting of the American Peanut Research and Education Society has provided for a cost effective annual assembly of most all the U.S. collaborators and a good representation of host country collaborators. Communications of research information and exchange of ideas has been facilitated through targeted workshops, such as the Peanut CRSP Workshop held in Khon Kaen Thailand in August 1986.

Networks among various research groups are used to maximize the extent and efficiency of resource utilization. Disciplinary networks within the CRSP, including U.S. and host country researchers, fosters interaction in areas such as breeding or entomology. Research is further enhanced by interdisciplinary exchanges, such as close cooperation between breeders and pathologists. A final level of networks brings together international groups with mutual interests. Close cooperation is maintained with ICRISAT in program planning and mutually supported projects (publication of the International Arachis Newsletter, a planned West Africa Regional Germplasm Evaluation network). The CRSP and IDRC mutually planned and supported research in Thailand. Interaction with ACIAR and ICRISAT has evolved in the peanut stripe virus research program. Cooperatively planned workshops and conferences with these and other groups enhances research communication.

### Networks

As indicated in the above strategy statement, several networks have evolved in the Peanut CRSP since its inception. These are maturing to the point of being rather effective in enhancing research programs and in dissemination of information developed.

#### A. CRSP AND ICRISAT

The ICRISAT peanut (groundnut) program began in 1976. CRSP planning activities began in 1980 with research implementation in 1982. Key peanut researchers around the world had professional interaction prior to 1976 which provided the embryo for a network enhanced by present CRSP, ICRISAT, and host-country programs involving many of the same individuals.

Close ICRISAT and CRSP cooperation began with ICRISAT Groundnut Program Leader, Ron Gibbons, enlisted as a member of a CRSP planning grant advisory panel. As a member of the

CRSP Board of Directors since 1982, Ron has been invaluable in helping maintain proper global focus to the CRSP. In his present position as Director of the ICRISAT Sahelian Center (ISC) in Niamey, Niger, Ron will continue on the Board and provide special insights to the CRSP West Africa program.

Strength through diversity provides a commonality between the CRSP and ICRISAT. ICRISAT goals and objectives are based on broad, regional or zonal constraints with minimal donor type resources to support national programs. The CRSP, although global or zonal in nature, is based on linkages with and direct donor type support to national programs. In addition to CRSP developed technology, the CRSP serves as a conduit for ICRISAT generated technology, resulting in a synergistic effect of the network.

The programs are studied and analyzed annually to seek areas for closer coordination and to minimize duplication.

The CRSP supports host-country researchers in appropriate ICRISAT training programs. Joint workshops and seminars have been held on such subjects as rosette virus, aflatoxins, and comprehensive regional meetings of groundnut workers.

We cooperate to publish the *International Arachis Newsletter*, which is accessible to scientists worldwide for communicating research results. The CRSP contributes \$5,000 per year toward publication costs.

The CRSP and ICRISAT are directly complementary in research programs. For example, in West Africa the CRSP has an comprehensive program in entomology, while ISC has only ten percent of the time of one entomologist on peanut.

#### B. CRSP and IRHO

The French support peanut research in West Africa through IRHO (Institute for Oilseeds Research). Pierre Gillier, former IRHO Director, served as an advisor to the CRSP planning effort and later was a member of the External Evaluation Panel.

IRHO staffs three scientists in Senegal that also receive CRSP support in a cooperative effort to enhance the Senegal research program. Cooperation exists with an IRHO scientist in Burkina Faso.

Frequent contact is made with IRHO staff in Paris by U.S. collaborators traveling to West Africa. Bocklee Morvan is the present IRHO Director.

IRHO publishes the journal *Oleagineux*, the primary journal for reporting peanut research results in West Africa.

#### C. CRSP and CARDI

CRSP and CARDI (Caribbean Agricultural Research and Development Institute) interaction began with the Planning Grant, 1980-1982. Dr. St. Clair Forde, CARDI, Director of Research served on the planning grant advisory panel.

A research Caribbean program was developed in the CRSP. Primary collaboration was through a linkage with CARDI. The CARDI network of some 13 English speaking countries has fostered research and dissemination of information in the Region.

D. An informal network of interested researchers in peanut virus research has greatly facilitated CRSP research in this area. The CRSP has been instrumental in linking research on rosette virus with CRSP researchers at the University of Georgia and Ahmadu Bello University in Nigeria, ICRISAT researchers in India and Malawi, Scottish Crops Research Institute, and the West German Virus Institute.

The collaboration in Scotland and Germany has been especially beneficial in that these countries do not produce peanuts, and researchers can take samples containing live virus to the laboratories for detailed analyses.

- E. Aflatoxin research has been fostered through informal networks involving CRSP, ICRISAT and host-country scientists.
- F. The Peanut CRSP and the International Development Research Centre-Canada (IDRC) has jointly support peanut research in Thailand for several years. The Thai program is an integrated effort involving the Department of Agriculture (DOA), Kasetsart University (KU) and Khon Kaen University (KKU). The CRSP has supported DOA, KU, and KKU. IDRC support goes mainly to KU and KKU. A coordinated, efficient, and productive program has been developed.
- G. A linkage with the Australian Centre for International Agricultural Research (ACIAR) has developed during the past year relative to the stripe virus program. The CRSP is developing a new initiative in Thailand and the Philippines, while ACIAR supports a program in Indonesia. Planning meetings have involved the four groups. Recent discussions at the Regional Groundnut Scientists Meeting held in November 1988 in Malang, Indonesia should lead to cooperation between the CRSP, ACIAR, Philippines, and Indonesia. The CRSP breeding program in the Philippines is developing acid tolerant peanut lines that should be used in the acid soil program supported by ACIAR in Indonesia and Malaysia.

The networks exist, are complementary, and are growing stronger. The networks exist in the institutions, and are personified in the individual researchers.

## Management Organization and Accomplishments

The University of Georgia is the Management Entity for the Peanut CRSP and received the grant from AID. Georgia subgrants to Alabama A&M University, University of Georgia, North Carolina State University, and Texas A&M University, that lead the research projects in collaboration with the host countries. A Board of Directors, Technical Committee, External Evaluation Panel, and AID personnel advise and guide the Management Entity in areas of policy, technical aspects, budget management, and review.

### *Management Entity*

#### Responsibilities

The University of Georgia Management Entity office is located in the College of Agriculture at the Georgia Station, Griffin, Georgia. The major role is responsibility to AID and the participating institutions for technical and administrative matters for the CRSP. Duties include negotiating agreements, fiscal management, progress reports, and project modification.

#### Organization

The Management Entity staff (CRSP financed) is comprised of:

- Dr. David G. Cummins, Program Director  
From April 22, 1988
- Dr. Tommy Nakayama, Program Director  
Through April 21, 1988
- Mrs. Barbara Donehoo, Administrative Secretary
- Mrs. Michelle S. Dillard, Accounting Assistant

**Supportive Management staff (non CRSP financed):**

Mr. Ted Proffer, Business Manager, University of Georgia,  
College of Agriculture  
Dr. Darl Snyder, Director of International Development and  
Title XII Representative, University of Georgia.

**Accomplishments**

- Provided support to Principal Investigators in project management, travel clearances, and equipment approval.
- Planned and facilitated two Board of Director's Meetings and coordinated one conference call.
- Planned and facilitated two Technical Committee meetings and coordinated one conference call.
- Arranged and conducted annual meeting of PI's following the APRES Meeting at Orlando, FL.
- Visited host country institutions in Southeast Asia for program consultations.
- Sponsored and participated in Peanut Utilization Workshop at the 7th World Congress of Food Science and Technology in Singapore. Participated in the International Workshop on Aflatoxin Contamination of Groundnut at the ICRISAT Center in India.
- Participated in CRSP Directors and Program Managers Workshop: "Decade Two Preparation" in Washington, DC.
- Participated in International Centers Week in Washington, DC.
- Published 1986/87 Annual Report.
- Ted Proffer participated in the Financial Management Seminar for Contract Officers and Program Directors in Washington, DC.

**Board of Directors**

**Responsibilities**

The Board of Directors serves in an advisory role to the Management Entity and provides liaison to their respective institutions. The duties of the Board of Directors are to establish policy for the CRSP, approve annual budgets, approve recommendations on programs, and review accomplishments of the CRSP.

**Organization**

The Board consists of one administrative representative from each of the participating U.S. institutions and one from ICRISAT for a total of five members. The length of term of members is at the discretion of the individual institutions. A chairman and secretary are elected. The present board members are:

Dr. Dudley T. Smith, Chairman  
Associate Director, Texas  
Agricultural Experiment Stations  
Texas A&M University

Dr. Charles W. Laughlin  
Associate Director, Georgia  
Agricultural Experiment Stations  
University of Georgia

Dr. B. Onuma Okezie  
Director of International Program  
Alabama A&M University

Dr. Billy E. Caldwell, Secretary  
Head, Department of Crop Science  
North Carolina State University

Dr. Ron W. Gibbons  
Director of ICRISAT Sahelian Center  
Niamey, Niger

**Accomplishments**

The Board of Directors met three times; in Orlando, FL in July; Atlanta, GA in December, and by conference call in June. Major activities were:

- Dr. Caldwell participated in CRSP Directors and Program Managers Workshop: “Decade Two Preparation” in Washington.
- Approved annual program plans and budgets.
- Approved modifications in budget during year.
- Reviewed and approved fifth year annual report.
- Approved modifications in three projects: (1) shift of Georgia Peanut Virus project from Nigeria to Philippines and Thailand, (2) Transfer of Alabama A&M Food Technology project from Sudan to Burkina Faso, and (3) conversion of Georgia Cultivar Improvement Project in the Caribbean to a Post Harvest Research Project.
- Reviewed and concurred with Economic Survey papers for West Africa developed by Dr. William Morris, Purdue University.
- Participated in selection and approval of nominees for the External Evaluation Panel.

### *Technical Committee*

#### Responsibilities

The Technical Committee acts in an advisory role to the Board of Directors and Management Entity. Primary duties are to review and recommend plans for research, training, and budgetary components of the projects, establish mechanisms for program coordination in host countries, and assist in planning annual reviews.

#### Organization

The committee consists of one principal investigator from each participating U.S. institution. The Technical Committee is:

Dr. Olin D. Smith, Chairman  
Department of Crop Science  
Texas A&M University

Dr. Bharat Singh  
Department of Food Science  
Alabama A&M University

Dr. Johnny C. Wynne  
Department of Crop Science  
North Carolina State  
University

Dr. Manjeet Chinnan  
Department of Food Science and  
Technology  
University of Georgia

The Program Director/Administrative Secretary of the University of Georgia Management Entity serves as secretary to the committee.

#### Accomplishments

The Technical Committee formally met three times; in Orlando, FL in July, Atlanta, GA in December and by conference call in June. The members individually advised the Board and Program Director on several occasions. Items of concern were:

- Evaluated principal investigator requests for budget modifications and forwarded recommendations to the Board of Directors.
- Prepared progress reports for Annual Report.
- Dr. Singh participated in CRSP Directors and Program Managers Workshop: “Decade Two Preparation” in Washington, DC.
- Recommended modifications in three projects: (1) shift of Georgia Peanut Virus project from Nigeria to Philippines and Thailand, (2) Transfer of Alabama A&M Food Technology project from Sudan to Burkina Faso, and (3) conversion of Georgia Cultivar Improvement Project in the Caribbean to a Post Harvest Research Program.
- Participated in selection and approval of nominees for the External Evaluation Panel.

### *External Evaluation Panel*

The External Evaluation Panel was described in the CRSP Plan to consist of three to five eminent scientists recommended by the Management Entity for review and approval by AID. Their role is to monitor and evaluate program direction and accomplishments. Duties include a review of projects and programs of the CRSP and provide written evaluation, and recommendation for addition, elimination, or modification of component projects and overall objectives to include retention, elimination, or addition of new overseas sites. A new Panel was selected by the CRSP for submittal to AID/BIFAD, since the present panel had served for the life of the CRSP. The consensus was that a new group should be formed and contact former members on a consultant basis when necessary for specific evaluations. Nominees submitted to AID/BIFAD were:

Dr. Johnny W. Pendleton, Adjunct Professor, University of Illinois  
 Dr. John P. Cherry, Director, USDA Eastern Regional Research Center  
 Dr. Allan J. Norden, Professor Emeritus, University of Florida  
 Dr. Ray O. Hammons, Peanut Geneticist/Breeder, Retired, USDA/ARS.

The EEP activities for the year were as follows:

- Dr. Ken Garren (USDA/ARS, Suffolk, VA, retired) attended the CRSP Directors and Program Managers Workshop: "Decade Two Preparation" in Washington, DC.
- Dr. Ken Garren reviewed and evaluated the Peanut CRSP program from the 1986/87 Annual Report and submitted a written report to the Management Entity. Dr. C. O. Chichester (University of Rhode Island) reviewed the Food Technology projects on a consultant basis.

### *Coordination with AID and BIFAD*

AID—Liaison is maintained with AID on a continuing basis. Dr. Loren Schulze, Science and Technology/Agriculture, USAID/Washington is the Peanut CRSP Project Manager. Activities during the year included:

- Continual advice in program management, direction, and development
- Coordination with Mission programs
- Securing travel approvals
- Clearance for equipment purchases
- Maintained communications with AID/Washington for various reports
- Attended CRSP collaborators meeting in Orlando, FL
- Attended Board of Directors and Technical Committee meeting in Orlando, FL and Atlanta, GA, and participated in conference call meeting
- Participated in CRSP Directors and Program Managers Workshop: "Decade Two Preparation" in Washington, DC
- Participated in Financial Management Seminar for Contract Officers and Program Directors in Washington, DC
- Participated in International Centers Week in Washington with Program Directors.

BIFAD—Advice is provided by BIFAD in various areas of concern in program development and management. The CRSP maintains a liaison with BIFAD.

Mr. William Fred Johnson is the BIFAD liaison to the CRSP. Dr. John Stovall attended the Collaborators, Board of Directors, and Technical Committee meetings in Orlando, FL, representing Mr. Johnson.

### *Peanut CRSP-ICRISAT Program Analysis/Coordination*

The CRSP Plan calls for an annual conference with appropriate ICRISAT personnel to analyze the peanut research programs of the two groups to avoid duplications or CRSP substitutions for ICRISAT responsibilities. Programs of both groups emphasize Semiarid Tropical regions and a common funding

source contributes to the need for such an analysis. Joint plans will insure maximum results from research efforts.

The analysis/coordination has evolved into an ongoing process rather than a singular effort.

Dr. Ron Gibbons, Director of the ICRISAT Sahelian Center and West African Programs with headquarters in Niamey, Niger is a member of the Board of Directors, which has been a most important asset in coordination. His presence on the policy making Board provides up-front advice on matters of mutual interest between the two groups. Correspondence by letter and telex supplement his involvement when he is not able to attend Board Meetings. Other areas of coordination were discussed earlier in the *Strategy* and *Networks* sections.

## Training

One objective of the Peanut CRSP is to improve the research capability of host country institutions by offering short term and degree oriented training programs for host-country staff at U.S. institutions and providing on-site consultation in the host countries by U.S. scientists. In addition, U.S. university phases of the research programs are enhanced through support of U.S. graduate students. Accomplishments during the year follow.

- A total of 13 host country scientists visited collaborators at the U.S. institutions on a scientist-to-scientist basis. Activities included:
  - a. Laboratory and field training in research methodologies.
  - b. Reviewing of research accomplishments.
  - c. Planning of future research activities.
- A total of ten host country scientists visited "third-country" scientists or institutions in various short term training programs or workshops.
- Twenty-five students supported by host country CRSP budgets were enrolled in programs leading to graduate degrees at NCSU, UGA, TAMU, and AAMU. Eleven completed studies during the year.
- Twenty-four students (22 U. S. and 2 foreign) were provided either full-time or partial support from the U. S. CRSP budgets for graduate degree programs at AAMU, UGA, TAMU, and NCSU.
- Fourteen U.S. scientists made various site visits during the year. Total time spent was 225 days or 0.86 man years. The scientists reviewed research progress, discussed and developed future research plans, participated in field and laboratory research, and provided training in specific field and laboratory research techniques. The budget reduction reflected in a decreased amount of time spent on overseas activities by U.S. scientists.

## Program Support

The Peanut CRSP grant from AID provided \$1,701,188 for the period 1 July 1987 to 30 June 1988. A total of \$1,334,319 was expended during the same period (\$1,100,053 program and \$234,266 Management Entity). The U.S. university cost-share contribution was \$377,980. Total Aid funds budgeted and expended for 1 July 1982 to 30 June 1988 was \$9,154,388 and 8,605,399 respectively. In addition the U.S. universities contributed \$2,389,505 for the 6-year period. (Table 1).

Compliance with the Gramm Rudman Hollings initiative has resulted in an 13.5% reduction in current funding following the 18% cut last year.

Cumulative expenditures for the Management Entity are shown in Table 2. All categories of the expenditures are less than the budgeted amounts.



**Table 1. Summary of sources of support budgeted and expended in the Peanut CRSP for 1982, 1983, 1984, 1985, 1986/87, and 1987/88.**

<u>Item</u>	<u>7/1/82-6/30/87</u>	<u>1987/88</u>	<u>TOTAL</u>
<b>BUDGETED</b>			
AID Program			
Cost Shared	4,264,027	895,184	5,159,211
Not Cost Shared	2,057,696	464,816	2,522,512
Total	6,321,723	1,360,000	7,681,723
Management Entity	1,131,477	341,188	1,472,665
Total	6,321,723	1,360,000	7,681,723
University Support (Cost share)	1,818,342	438,038	2,256,380
Grand Total	9,271,542	2,139,226	11,410,768
<b>EXPENDED</b>			
AID Program			
Cost Shared	4,380,769.*	809,466	5,190,235
Not Cost Shared	1,838,072	290,587	2,128,659
Total	6,218,841	1,100,053	7,318,894
Management Entity	1,052,239	234,266	1,286,505
Total	7,271,080	1,334,319	8,605,399
University Support (Cost share)	2,011,825	377,980	2,389,805
GRAND TOTAL	9,282,905	1,712,299	10,995,204

\* 3921 was added to previously reported year ending June 30, 1987.

Table 2. Allocation and Expenditure of Program Funds for 1987/88.

Project	US	BUDGETED AID FUNDS		Univ. Cost Share
		HC	Total	
AAM/FT/CAR	33,884	16,116	50,000	10,095
AAM/FT/S	65,826	24,174	90,000	20,000
GA/FT/TP	74,651	40,349	115,000	18,137
GA/IM/BF	59,250	19,750	79,000	44,651
GA/PH/CAR	65,000	60,000	125,000	16,250
GA/PV/N	48,760	34,240	83,000	60,485
NCS/BCP/TP	132,000	126,000	258,000	44,000
NCS/IM/TP	60,000	25,000	85,000	20,000
NCS/SM/TP	50,000	0	50,000	16,667
TX/SM/TP	50,000	0	50,000	16,650
TX/BCP/S, BF, N	162,000	81,000	243,000	54,000
TX/MM/S	93,813	38,187	132,000	34,000
<b>TOTAL</b>	<b>895,184</b>	<b>464,816</b>	<b>1,360,000</b>	<b>354,935</b>
<u>EXPENDED</u>				
(7/1/87-6/30/88)				
AAM/FT/CAR	30,410	11,908	42,318	4,634
AAM/FT/S	63,256	34,792	98,048	7,191
GA/FT/TP	67,203	35,787	102,990	58,813
GA/IM/BF	50,728	3,151	53,879	33,250
GA/PH/CAR	38,014	15,273	53,287	16,635
GA/PV/N	52,794	25,284	78,078	541
NCS/BCP/TP	117,225	107,530	224,755	44,000
NCS/IM/TP	60,532	22,150	82,682	20,000
NCS/SM/TP	69,788	0	69,788	16,667
TX/SM/TP	32,575	-0-	32,575	32,358
TX/BCP/S	141,013	22,564	163,577	86,693
TX/MM/S	85,928	12,148	98,076	57,198
<b>TOTAL</b>	<b>809,466</b>	<b>290,587</b>	<b>1,100,053</b>	<b>377,980</b>

**Table 3. Cumulative Budgets and Expended Program Funds from 7/1/82 thru 6/30/88**

	<u>BUDGETED</u>			Cost Share
	US	HC	Total	
AAM/FT/CAR	261,734	103,015	364,749	66,199
AAM/FT/S	399,115	176,593	575,708	135,991
GA/FT/TP	260,583	243,951	504,534	136,803
GA/IM/BF	290,924	119,039	409,963	191,180
GA/PH/C	346,969	206,431	553,400	273,249
GA/PV/N	375,731	145,355	521,086	288,498
NCS/BCP/TP	677,667	603,901	1,281,568	206,023
NCS/IM/TP	294,362	151,816	446,178	166,529
NCS/SM/TP	436,379	242,121	678,500	163,613
TX/SM/TP	375,592	42,081	417,673	116,083
TX/BCP/S	825,180	300,208	1,125,388	308,076
TX/MM/S	614,975	188,001	802,976	201,122
<b>Total</b>	<b>5,159,211</b>	<b>2,522,512</b>	<b>7,681,723</b>	<b>2,253,366</b>
	<u>EXPENDED</u>			
	(Inception thru 6/30/88)			
AAM/FT/CAR*	264,085	71,628	335,713	74,074
AAM/FT/S	378,983	177,198	556,181	84,261
GA/FT/TP	285,469	220,348	505,817	214,882
GA/IM/BF	287,204	71,814	359,018	207,438
GA/PH/CAR	320,113	146,846	466,959	326,407
GA/PV/N	415,709	93,932	509,641	288,074
NCS/BCP/TP	668,845	579,478	1,248,323	240,086
NCS/IM/TP	298,046	136,317	434,363	98,337
NCS/SM/TP	444,718	253,560	698,278	185,366
TX/SM/TP	400,246	-0-	400,246	163,683
TX/BCP/S	814,285	221,475	1,035,760	329,408
TX/MM/S	612,532	156,062	768,594	206,408
<b>Total</b>	<b>5,190,235</b>	<b>2,128,658</b>	<b>7,318,893</b>	<b>2,418,424</b>

\* 3921 was added to previously reported year ending June 30, 1987.

Table 4. Cumulative Management Entity costs and Expenditures for 1982, 1983, 1984, 1985, 1986/87 and 1987/88

Item	<u>BUDGETED</u>		Total
	1982-1986/87	1987/88	
Salaries	390,000	140,000	620,000
Staff Benefits	90,000		
Supplies and Equipment	29,500	8,600	38,100
Travel*	104,000	15,000	119,000
Communication	30,000	8,600	38,600
Meeting Costs**	50,000	20,000	70,000
International Arachis Newsletter	25,000	5,000	30,000
Contract Studies	170,000	45,885	215,885
Technical Assistance	50,000	15,000	65,000
TOTAL	938,500	258,085	1,196,585
ME Indirect Costs	286,243	83,103	369,346
Sub Contract Indirect	83,875	0	83,875
Supplement, overseas audit	88,84		788,847
Overall TOTAL	1,397,465	341,188	1,738,653
	<u>EXPENDED</u>		
	(thru 6/30/88)		
Salaries	382,429	100,913	483,342
Staff Benefits	85,002	24,036	109,038
Supplies and Equipment	25,561	3,860	29,421
Travel*	55,600	11,984	67,584
Communication	27,532	2,002	29,534
Meeting Costs**	133,159	18,057	151,216
International Arachis Newsletter***	-0-	10,000	10,000
Contract Studies****	24,451	8,662	33,113
Technical Assistance	8,307	0	8,307
TOTAL	742,041	179,514	921,555
ME Indirect Costs	226,323	54,752	281,075
Sub Contract Indirect	83,875	0	83,875
Overall TOTAL	1,052,239	234,266	1,286,505

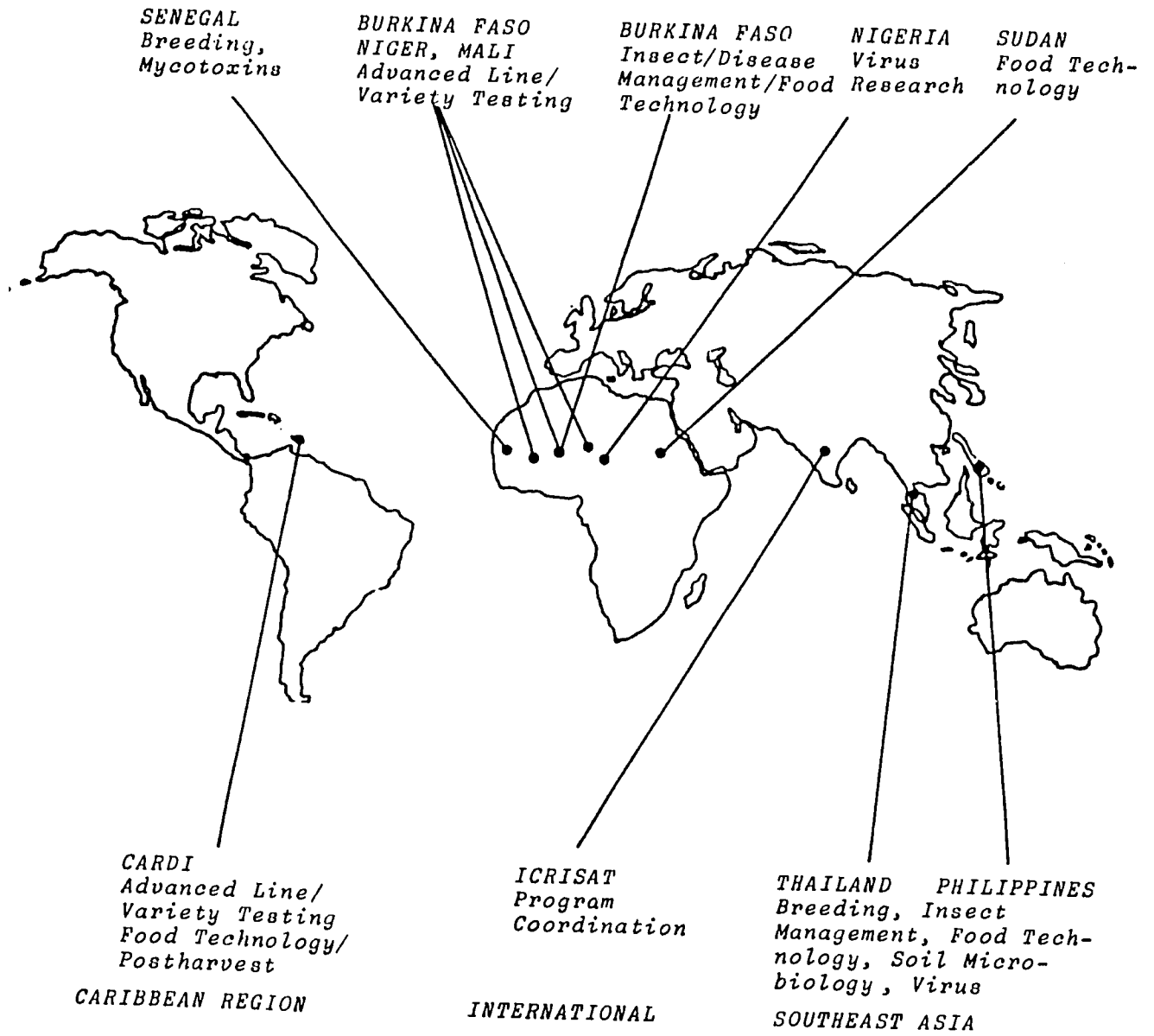
\* ME International Travel

\*\* ME Domestic, Board, TC and EEP Travel

\*\*\* For Years 1986/87-1987/88

\*\*\*\* Thailand audit-\$8162, C. O. Chichester \$500

*Project Annual Reports: FY 1987/88*  
**GLOBAL NATURE OF PEANUT CRSP**  
**SEMIARID TROPICAL AFRICA**



## Project Annual Reports

### Introduction

The Peanut CRSP has active projects in three world regions, Semiarid Tropical Africa, Southeast Asia, and the Caribbean. A wide range of disciplines are covered in the country programs in the six African countries, two Asian countries, and the Caribbean, (primarily in four countries) which increases the potential exchange of information on regional and interregional basis. All the locations are within the area bounded the latitudes 11° and 17° north.

Research agreements were completed in all the countries before or during FY 84. Research progress was significant in most projects, but variations occurred because of a number of factors affecting rate of startup of research in the host countries.

The annual progress reports were prepared by the U. S. Principal Investigators. Results presented are from research accomplished by both the U. S. and host country collaborators. Space available has limited the detail of presentation in many instances. If a reader would like more information related to a particular project, please contact the Principal Investigator or the Management Office.

Progress in the Peanut CRSP is a reflection of the interest that the U. S. and host country researchers have in the different areas of research, the rapport developing because of the collaborative mode, commitment to the development process, and the administrative support provided.

#### *Breeding —*

TX/BCP/S,BF,N: Disease-Resistant Peanut Varieties for Semi-Arid Environments

NCS/BCP/TP: Peanut Varietal Improvement for Thailand and The Philippines

#### *Mycotoxin —*

TX/MM/S: Mycotoxin Management in Peanut by Prevention of Contamination and Monitoring

#### *Pest Management —*

GA/IM/BF: IPM Strategies for Peanut Insects in SAT Africa

NCS/IM/TP: Management of Arthropods on Peanuts in Southeast Asia

GA/PV/N,TP: Peanut Viruses: Etiology, Epidemiology and Nature of Resistance

#### *Soil Microbiology —*

NCS/SM/TP: Influence of Soil Microbiology on Nitrogen Fixation and Growth of Peanuts in Thailand and Philippines. A. Rhizobia

TX/SM/TP: Influence of Soil Microbiology on Nitrogen Fixation and Growth of Peanut in Thailand and the Philippines. B. Mycorrhiza

#### *Food Technology/Post Harvest —*

AAM/FT/S,BF: An Interdisciplinary Approach to Optimum Food Utility of the Peanut in SAT Africa

GA/FT/TP: Appropriate Technology for Storage/Utilization of Peanut

AAM/FT/CAR: Peanut Utilization in Food Systems of Developing Countries

GA/PH/CAR: Postharvest Handling Systems for the Small Peanut Producer.

TX/BCP/S,BF,N

**Disease-Resistant Peanut Varieties  
for Semi-Arid Environments****Texas A&M University  
Institut Senegalais de Recherches Agricoles  
University of Ouagadougou Institut Superior Polytechnique  
Institut Nationale de Recherches Agronomiques du Niger**

O.D. Smith, Principal Investigator

J.C. Mortreuil (Senegal); P. Sankara (Burkina Faso); A. Mounkaila (Niger);  
A.M. Schubert, C.E. Simpson, and D.H. Smith (TAMU)—Co-Investigators**1 INTRODUCTION**

The climate and land resources in the developing countries of Sahelian West Africa present major challenges to peanut production. Short rainy seasons with intermittent periods of drought coupled with soils with little organic matter, low fertility, and low moisture holding capacity make predictable yields problematical. These stresses contribute to the threats from diseases, mycotoxin-producing fungi, and arthropods. Stable varieties, buffered against these major production constraints, yet yielding good quantities of wholesome, acceptable products, are the best and most cost-efficient approach to relieving hunger in this region. The development of high yield potential varieties with tolerance or resistance to these constraints can increase the quality of living in Sahelian West Africa.

Rainfall during the 1987 cropping year in West Africa varied greatly from location to location. At Tarna, Niger, less than 200 mm of rain fell during the growing season. As in 1986, the rains began very late at Bambey, Senegal (July 19). Total rainfall at Bambey and Niore, Senegal was 365 and 902 mm, respectively. The southwestern peanut growing areas of Burkina Faso received adequate rainfall; Niangoloko received 973 mm from April through October, and Bobo-Dioulasso received 681 mm. Weekly rainfalls of record at; Niangoloko and Bobo-Dioulasso, Burkina Faso, Bambey and Niore, Senegal, and at the Tarna Experiment Station near Maradi, Niger, are provided in Figures 1 through 3, respectively. Cultural practices were similar for the evaluations in West Africa reported herein. No irrigation, pesticides, insecticides, or fungicides were used except as noted. Weeds were hand controlled. Locations in Burkina Faso were fertilized at planting. Soil types included fine sand at Tarna, Niger and Niangoloko, Burkina Faso, fine clayey sand at Gampela, Tenkodogo, and Bobo-Dioulasso, Burkina Faso, and sandy clay at Saria, Burkina Faso.

Figure 1. Rainfall, 1987, Southwest Burkina Faso

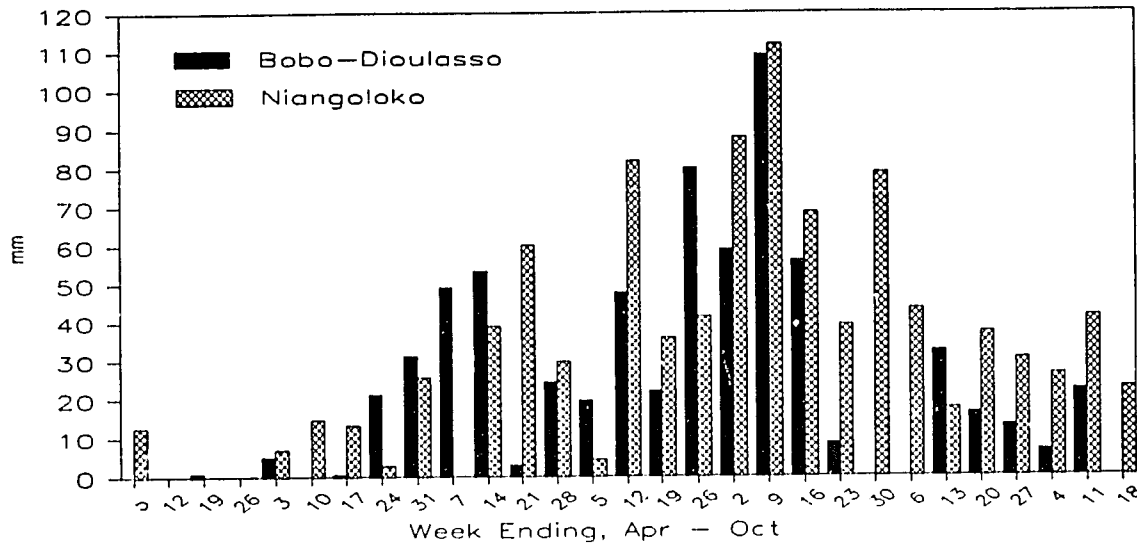
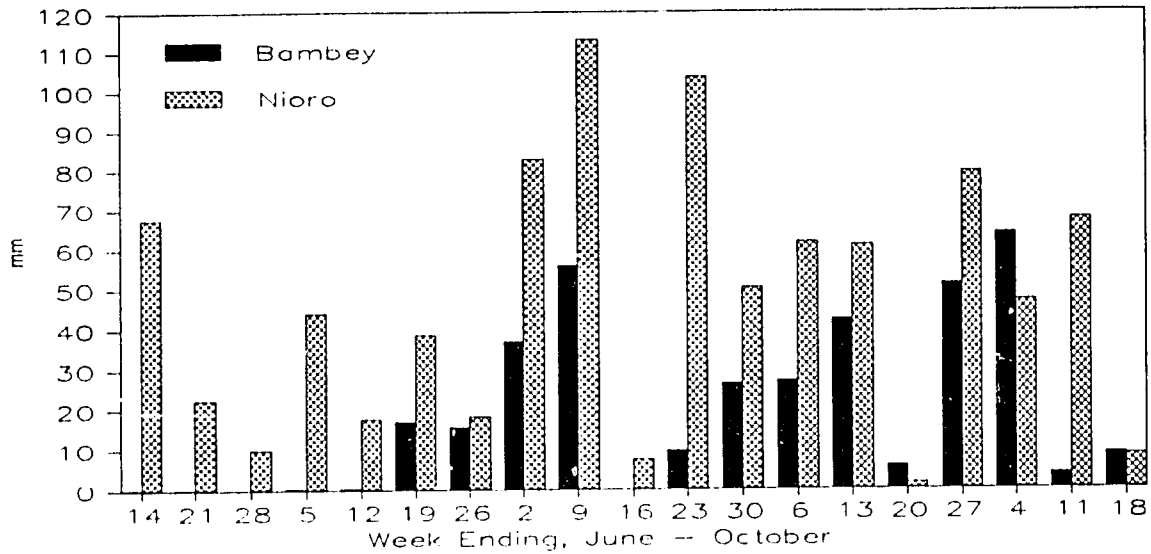


Figure 2. Rainfall, 1987, Bambey and Nioro, Senegal



In Texas, little rainfall occurred in July, necessitating irrigation at all locations except at Waller, where irrigation was not available. Figure 4 provides weekly totals of water added to the soil from rainfall and irrigation, at Bryan and Yoakum. Rainfall data for Waller, Wilson, and Frio Counties were not available.



Figure 3. Rainfall, 1987, Tarna, Niger

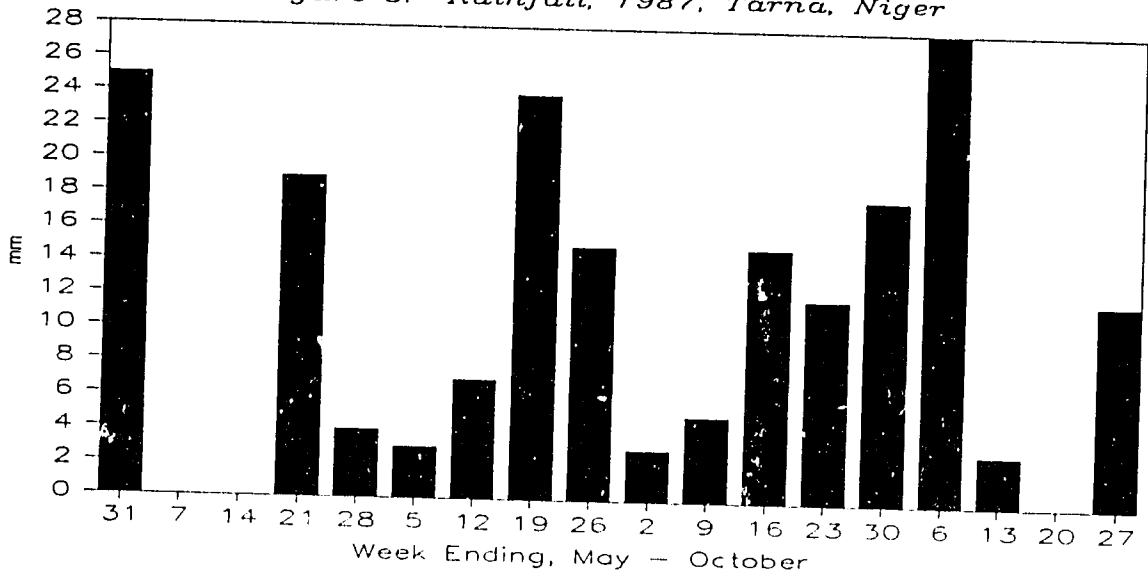
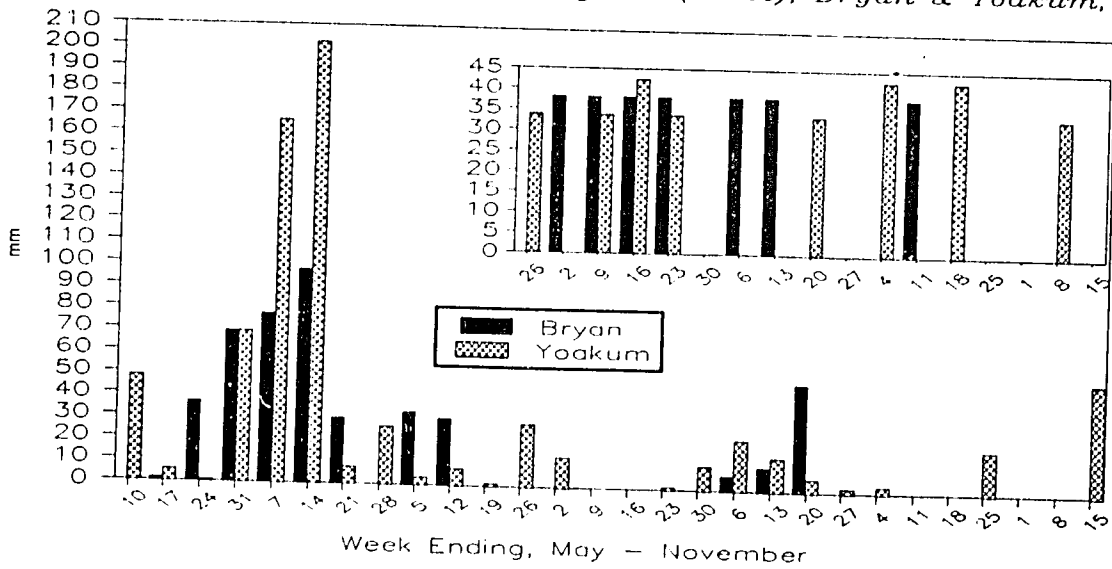


Figure 4. 1987 Rainfall and Irrigation (inset), Bryan & Yoakum, TX



Cultural practices were similar at most locations. At Yoakum, 812 kg/ha of 14-6-6 fertilizer was applied to the ryegrass cover crop in the Fall of 1986, while at Bryan, 280 kg/ha of 19-19-19 was applied December 3, 1986 to the cover crop, and 336 kg/ha of 19-19-19 was applied March 9, 1987 prior to planting. Pre-emergence herbicides were used at all locations for weed control. Where leafspot infection was not evaluated, foliar diseases were controlled with fungicides applied on a weekly or biweekly basis. Gypsum was added twice to the soil at Bryan; July 11 at the rate of 442 kg/ha, and August 26 at 560 kg/ha.

Statistical analyses were performed with the Statistical Analysis System GLM or ANOVA procedures. Significance levels were  $p=0.05$  unless otherwise stated.

In Texas and in the host countries of West Africa, the objectives of this Peanut CRSP project overlap with those of on-going projects. Expenses associated with research supporting total programs, such as labor, fuel, repairs, and supplies are funded with both CRSP and non-CRSP funds. This report attempts to emphasize research clearly addressing the objectives of this CRSP project. No attempt has been made to include all research conducted at any of the participating institutions.

## 2 MAJOR ACCOMPLISHMENTS

Average yield in plots at Niangoloko, Burkina Faso treated with insecticide and nematicide was 149% greater than that in the untreated test.

Five entries grown in both 1986 and 1987 at Niangoloko were highly resistant to rust, scoring 3.0 on the ICRISAT scale in both years. Among these five lines were Tx855157 and Tx855101, two lines derived from a cross of TxAG-3 and Tamnut 74 and selected for pod rot resistance.

In replicated evaluations of ICRISAT germplasm in Texas, PI390593 was rated significantly lower in mixed early and late leafspot than Southern Runner and yielded 110% of Southern Runner and 123% of Florunner.

Four entries, RCM753, RCM754, RCM755, and RCM757 had the lowest ICRISAT leafspot scores at all three rating dates (3,5,5 at 85, 106, 116 DAP respectively) in contrast to the most resistant check, Southern Runner, which averaged 3.9, 5.3, and 6.3 at the three respective rating dates.

Over three years of testing at Bambey, Senegal, PI 1174 exceeded pod yields of local checks by almost 20%.

Several selections from a cross of 57-422 x Samaru 1064 yielded more than 3000 kg/ha in yield trials at Nioro, Senegal.

Under dryland conditions in Texas, the selection J-11/Robut33-1 averaged 113% of Pronto for pod yield, and 109% of Pronto for value per hectare for 1986 and 1987.

In comparisons of early ICRISAT germplasm under dryland conditions in Texas during the 1986 and 1987 seasons, ICGS(E)-56 averaged 132% and 128% of Starr for pod yield and value/ha, respectively.

Data from a complete diallel involving five early maturing cultivars, Chico, Sn55-437, TxAG-1, TxAG-2, and Tx851856, and the parental, F<sub>1</sub>, and F<sub>2</sub> generations studied in 1987 in four replications indicated Chico/Tx851856 and its reciprocal had the highest heritability for both flowering pattern and number of full or mature pods. Transgressive segregates earlier in flower setting and number of both full and mature pods were identified. The low correlations between pod parameters and flowering pattern indicated flowering pattern, particularly days to first flower, could not be effectively used to measure earliness in the early-maturing germplasm evaluated. The number of full-size pods and number of mature pods at harvest appeared to be better criteria for selecting early germplasm. No significant differences in general or specific combining ability were found for parents or single crosses.

In five replicated tests involving entries from the U.S., North Carolina, Florida and ICRISAT to evaluate yield and reaction to Tomato Spotted Wilt Virus (TSWV), several entries had lower disease and/or higher yields than test checks.

Disease surveys were conducted in Niger and Burkina Faso during the 1987 growing season. Leafspot was the primary foliar pathogen of importance, while rust was also found in more humid regions of Burkina Faso. Pod rot was economically important in the drought affected areas of Niger.

## 3 GOAL

Develop and identify peanut lines adapted to important ecological areas of Sahelian West Africa and Texas that are resistant to pathogens causing economic loss and effectively utilize available water, and to identify cultural practices that will maximize the yield potential of cultivars in those environments.

## 4 EXPECTED IMPACT OF PROJECT

Important constraints to peanut production in West Africa and in the Southwestern United States relate to drought and disease. Increased germplasm acquisition, recombination, evaluation, and selection

in varied host country and Texas environments coupled with improved methods for assessing and selecting superior lines will enable the development of better varieties with earliness, disease resistance, and/or drought tolerance that are adapted to the important peanut producing areas of these regions.

## 5 APPROACH

1. Evaluate Texas and Senegal breeding lines and cultivars under varied environments and disease pressures in Senegal and Texas to ascertain their relative productivity under varied conditions, and to identify genotypes with adaptations to specific environments of particular importance.
2. Evaluate the disease reaction of selected germplasm lines and checks in areas of Burkina Faso with relatively high rainfall where disease pressure is conducive to effective evaluation and screening.
3. Hybridize selected germplasm lines and cultivars, and pre-screen for important agronomic traits, disease reaction, and other relevant features in Texas to develop populations in which useful selection can be implemented at collaborating country test sites.
4. Acquire, evaluate, and utilize germplasm from ICRISAT and other peanut research organizations that is of benefit in the development of improved varieties.
5. Develop and employ screening techniques that will expedite the selection of peanut lines with drought tolerance and resistance to disease.
6. Evaluate select lines and germplasms from this and other breeding programs at diverse sites in Burkina Faso and Niger to ascertain usefulness as cultivars or parents.
7. Provide training, encouragement, and assistance to host country personnel for the development of effective host country peanut improvement programs.

## 6 ORGANIZATION

### 6.1 U.S. Lead Institution: Texas A&M University (TAMU)

<b>Principal Investigator</b>	Dr. Olin D. Smith, Department of Soil and Crop Sciences (S&CS), TAMU, College Station (CS)
<b>Co-Principal Investigators</b>	Dr. Charles E. Simpson, TAMU Research and Extension Center, Stephenville Dr. Donald H. Smith, TAMU Plant Disease Research Station (PDRS), Yoakum
<b>Cooperators</b>	Dr. A. Michael Schubert, TAMU PDRS, Yoakum Dr. Robert E. Pettit, Department of Plant Pathology and Microbiology, TAMU, CS Mrs. Ruth A. Taber, Department of Plant Pathology and Microbiology, TAMU, CS
<b>Research Scientist</b>	Mr. W. J. Grichar, TAMU PDRS, Yoakum
<b>Research Associates</b>	Dr. Gregory B. Parker, Dept. of S&CS, TAMU, CS Mr. Richard Davis, TAMU PDRS, Yoakum Mr. A. J. Jaks, TAMU PDRS, Yoakum
<b>Research Assistants</b>	Mr. Serafin M. Aguirre, Dept. of S&CS, TAMU, CS Mr. Ousmane N'Doye, Dept. of S&CS, TAMU, CS Ms. Lisa Wildman, Dept. of S&CS, TAMU, CS Mr. Dalton Durio, Dept. of S&CS, TAMU, CS
<b>Technician</b>	Mr. John Scherlen, TAMU PDRS, Yoakum
<b>Budget Analyst</b>	Ms. Tracie Robertson, TAMU Research Foundation
<b>Institutional Representatives</b>	Dr. Dudley T. Smith, Associate Director, Texas Agricultural Experiment Station, TAMU Dr. E.C.A. Runge, Head, Dept of S&CS, TAMU, CS

## 6.2 Senegal: Institut Senegalais de Recherches Agricoles (ISRA)

Director Generale	Dr. Moctar Toure, ISRA, Dakar
Directeur du Departement Production Vegetales	Dr. Francois Faye, ISRA, Dakar
Project Group Leader	Mr. J. C. Mortreuil, IRHO/CNRA/ISRA, Bambey
Collaborators	Mr. Daniel Annerose, IRHO/CNRA/ISRA, Bambey Mr. Jean L. Khalfoui, IRHO/CNRA/ISRA, Bambey

## 6.3 Burkina Faso: University of Ouagadougou Institut Superior Polytechnique (ISP)

Directeur des ISP	Dr. Guillaume K. Sessouma, ISP, Ouagadougou
Collaborator	Dr. Philippe Sankara, ISP, Ouagadougou

## 6.4 Niger: Institut Nationale de Recherches Agronomiques du Niger (INRAN)

Director Generale	Dr. Idrissa Soumano, INRAN, Niamey
Collaborator	Mr. Amadou Mounkaila, INRAN, Maradi

## 7 ACCOMPLISHMENTS IN DETAIL

### 7.1 West African Peanut Evaluation Program (WAPEP)

#### 7.1.1 Multiplication Tests

Thirty-two entries were sent to Niger and Burkina Faso prior to the 1987 cropping season. Germplasm was weighted toward earliness and/or leafspot resistance and was mostly spanish in type. Preliminary observations were made, although the primary function of these tests was seed increase.

Under the severe drought in Niger, three U.S. entries and three ICRISAT lines yielded over 400 kg/ha of pods, compared to the best West African check, "796", which yielded an average of 382 kg/ha. Under irrigated culture in Burkina Faso, yields of runner entries generally exceeded the spanish.

#### 7.1.2 Single Location Evaluations

##### 7.1.2.1 Niger

Thirty-one entries from 1986 WAPEP, one entry (Tainan 9) from 1985 INPEP, and three local checks were evaluated in an unreplicated experiment at Tarna. Two plantings were made, and each plot was four 15 m rows. The first (S1) was planted June 22 and harvested September 23. The second (S2) was planted July 8 and harvested October 13. The three checks, Sn55-437 (drought tolerant), 796 (early), and TS 32-1 (full season) were replicated four times.

The lack of adequate rainfall during the 1987 growing season severely depressed yields at both plantings. Only those entries listed below exceeded 400 kg/ha of pods or 200 kg/ha of seed in the first planting:

Entry	First Planting		Second Planting	
	Pods	Seeds	Pods	Seeds
Tainan 9	433	164	113	41
Tamnut 74	429	170	182	83
Tx798736	427	218	184	76
EC-5	408	170	147	67
CV 35	397	229	154	82
Sn 55-437	440	212	139	66
796	392	213	164	82
TS 32-1	281	120	134	52

Interestingly, the same entries, except Tainan 9, were also superior in the second planting. In 1986 multiplication evaluations, the same entries except CV 35 ranked in the top ten for seed yield.

#### 7.1.2.2 Burkina Faso

Thirty-two entries and TS 32-1, the local check, were planted at Gampela July 8 and harvested October 30. Plots consisted of two 3 m rows spaced 40 cm apart arranged in randomized complete blocks in six replications. Twenty of the thirty-two entries exceeded TS 32-1 in yield, although considerable variability among replications and entries was observed.

#### 7.1.3 Multiple Location Evaluations

##### 7.1.3.1 Niger

Two entries, O-20 (WAPEP 86) and Tainan 9 (LNPEP 85) with local varieties 796, TS 32-1, and Sn55-437 were evaluated at Tarna and Magaria (eastern Niger). At Tarna, both entries had higher pod yields than TS 32-1 and Sn55-437. At Magaria, Tainan 9 had a higher pod but lower seed yield than Sn55-437.

Seed of four varieties, T177-83, JL-24, Tainan 7, and Sn55-437 were given to farmers in two villages, Kodorogo and Guidan Tagno. Normal cultural practices were followed. Pod yield in kg/ha were as follows:

Village	T 177-83	JL-24	Tainan 9	Sn55-437	Mean
Kodorogo	1042	938	917	764	915
Guidan Tagno	500	500	458	416	469
Mean	771	719	688	590	

T 177-83, a selection from a cross of 55-437 and Chico, yielded high, comparatively, at all locations. While Tainan 9 and JL-24 performed better than Sn55-437 in pod yield, both had thick shells. These varieties have performed well in previous tests when moisture was not as restricted.

##### 7.1.3.2 Burkina Faso

Forty-five entries and one local check variety were grown in six replications of a randomized complete block design at five locations. Planting and harvest dates at Bobo-Dioulasso, Gampela, Niangoloko, Saria, and Tenkodogo were, respectively: June 30 and October 18; July 8 and October 26; June 26 and October 13; July 9 and October 29; and, July 7 and October 16.

Plots consisted of two 3 m rows 40 cm apart. Entries included all lines evaluated in the 1986 multi-location test in Burkina Faso, as well as 16 of 30 lines from the 1986 Gampela-only yield trial. The local check at Bobo-Dioulasso and Niangoloko was RMP 12, and at the other locations, TS 32-1.

Mean pod yields over entries at Gampela were well above those at other locations. Saria yields were next highest, followed in order by those at Bobo-Dioulasso, Tenkodogo, and Niangoloko. However, the yields reported in 1987, as well as in 1985 and 1986, were "calculated" using the average yield of plants surviving at harvest multiplied by the number of seeds planted less the four end plants. Compensation likely inflated projected yields in plots with reduced stands. Unadjusted data will be reanalyzed when available.

At Gampela, ten entries significantly out-yielded the check TS 32-1:

Entry	Pod Yield (kg/ha)	Entry	Pod Yield (kg/ha)
NC18226	8662 a	ICGS 30	6907 b-e
Va Bunch 67	7792 ab	ICGS 23	6678 b-f
ICGS 54	7593 ab	ICGS 24	6358 b-g
ICGS 17	7155 a-c	UF81414	6206 b-h
ICGS 21	7082 a-d	TS 32-1	4379 i-m

At Saria, GFA-2 yielded significantly more pods per hectare than any other entry. UF81414 and NC18226 also performed exceptionally. A total of seven lines had significantly higher yields than TS 32-1:

Entry	Pod Yield (kg/ha)	Entry	Pod Yield (kg/ha)
GFA-2	5404 a	Va Bunch 67	3492 b-d
UF81414	3968 b	UF812351	3253 c-e
NC18226	3946 b	ICGS 27	3150 c-f
ICGS 54	3614 bc	TS 32-1	2412 g-l

At Bobo-Dioulasso, the check variety RMP 12 yielded significantly more pods than any other variety in the test. However, three entries were noteworthy as shown:

Entry	Pod Yield (kg/ha)	Entry	Pod Yield (kg/ha)
RMP 12	4251 a	Va Bunch 67	3031 bc
NC18222	3378 b	NC18226	2842 cd

Fourteen entries had significantly higher pod yields than TS 32-1 at Tenkodogo. Five entries, headed by Sunrunner, were in the top statistical grouping:

Entry	Pod Yield (kg/ha)	Entry	Pod Yield (kg/ha)
Sunrunner	2505 a	Early Runner	2332 a-d
ICGS 30	2417 ab	Va Bunch 67	2221 a-e
Florunner	2359 a-c	TS 32-1	1610 m-v

Niangoloko plots were affected by rosette. Early Runner yielded significantly more pods than any other entry. Seventeen lines were not significantly different from RMP 12.

Certain test entries performed well over several locations. Comparisons involving Niangoloko used the treated plots (see section below), since results were more typical of other locations. Va Bunch 67 ranked in the top 20% at each location, and was the best overall entry tested in 1987. NC18226 ranked first at Gampela, and second at Saria and Bobo-Dioulasso. Only at Niangoloko was it not in the top 25% of entries. ICGS-54, -21, and -27 ranked in the top 20% at all locations except Bobo-Dioulasso, where they ranked at least in the upper half of entries. ICGS-30 and Sunrunner were in the top 20% at all locations except Saria and Gampela, respectively. Strangely, at Saria, ICGS-30 ranked next to last in yield.

#### 7.1.4 Niangoloko, Burkina Faso Pesticide Test

In past years, Niangoloko plots have been severely damaged by nematodes and aphid-transmitted rosette virus. To assess the efficacy of chemical control, two sets of plots of the 1987 WAPEP entries were planted. One set of plots was not treated with pesticides, while on the second set, the

pyrethroid insecticide delta-methrine (Decis) was applied July 30 and August 12 and the nematicide isazophos (Mural) was applied July 2, before planting. Planting and harvesting dates for the untreated plots were reported above. The treated plots were planted July 4 and harvested October 20. Experimental design and plot size were as previously described for WAPEP tests.

Average yield in the treated test was 149% greater than that in the untreated test. Rosette appeared to be the major constraint, as nematode injury symptoms were few even in the untreated test. However, other less obvious effects of the pesticides might have occurred. Certain entries appeared to benefit to a greater degree from treatment, although the statistical design used precludes making direct comparisons. ICGS-21 ranked 25th among entries in untreated plots, yet ranked first among entries in treated plots. Conversely, some lines did comparatively well under either set of conditions. Sunrunner, Early Runner, Florunner, and ICGS-27 were in the top 25% of entries in both tests.

### 7.1.5 Multi-Year Results, Burkina Faso

Peanuts are grown in climatically diverse regions of Burkina Faso, encompassing Bobo-Dioulasso and Niangoloko (high rainfall), through the drier central regions, represented by the stations at Tenkodogo and Saria, to Gampela, with the lowest rainfall of the five sites tested. RMP 12 is the major variety grown in the southwest regions, while TS 32-1 is the major cultivar of the rest of the peanut growing areas. In evaluating potential replacements for these two cultivars, several years of data is required to assure consistency of yield. Ideally, the potential new standard cultivar would do better under all conditions than the old variety, and the minimum for its consideration would be that it does no worse under marginal environments. Seed yield was considered an important criteria, since it included both pod yield and seed fill, and was considered as more reflective of the useable yield. Because grade data were not replicated, statistical analysis of seed yield data was not possible; only pod yield data were analyzed statistically. Both criteria were used in the discussion.

#### 7.1.5.1 1986 - 1987

Data for fifteen ICRISAT lines, four Senegalese lines, and the local check in tests at five locations in both 1986 and 1987 were analyzed for yield stability in multiple environments. Experimental design and plot layout were similar at all locations and in both years, although the number of entries tested differed between years.

At Bobo-Dioulasso, RMP 12 had the highest pod and seed yield over the two year period, primarily due to poor yields of other entries in 1987. ICGS-23 ranked second overall.

Severe rosette infection in 1986 at Niangoloko restricted yields of all entries except RMP 12 which has resistance to the virus. A slight pod yield advantage over RMP 12 was shown in 1987 by three of the ICRISAT lines, -11, -44, and -2. Because of the endemic nature of rosette, however, further testing of these lines at Niangoloko seems unwarranted.

ICGS-17, -30, -21, and -27 produced more seeds and pods than the check variety TS 32-1 at Gampela in both 1986 and 1987. No grade data were available for ICGS-54, the highest yielding entry in 1987; it yielded ninth in 1986, but with %TK of only 54%, ranked last in seed yield. 1987 grade data for ICGS-27 were also unavailable. This entry ranked third in pod yield and first in seed yield in 1986, and ranked fourth in pod yield in 1987. The yield of ICGS-16 was also noteworthy.

TS 32-1 performed better at Saria relative to the other entries than it did at Gampela. Only two entries, ICGS-3 and -27 had higher seed and pod yields in both years. ICGS-16 and -23 were better than TS 32-1 in pod yield in both years, but had lower seed yields.

At Tenkodogo, ICGS-27 ranked highest over both years in seed yield. Other entries that markedly out-performed TS 32-1 included ICGS-44, -16, -30, and -54.

Considering the three drier locations in which these tests were performed, Saria, Gampela, and Tenkodogo, ICGS-27 appeared to perform best at all locations. Other entries which appeared to do well in most locations were ICGS-54, -21, and -16. These entries, as well as those that yielded well at individual locations in both 1986 and 1987 should be tested further.

### 7.1.5.2 1985 - 1987

Ten U.S. cultivars, including four spanish, three runner, one virginia, and two valencia, and the local check were evaluated in tests at five locations in Burkina Faso from 1985 through 1987. Experimental design and plot layout were similar at all locations in all years, although the number of entries tested differed from year to year. These entries were part of a larger variety trial in all years.

At Bobo-Dioulasso, 1985 and 1986 could be characterized as "spanish" years; three of the four entries producing higher seed and pod yield than the bunch-type RMP 12 were spanish or valencia. The fourth variety, Early Runner, a runner type, yielded more in both years than RMP 12, as did Pronto and Tamnut 74. In 1987, however Sunrunner, Florunner, and Va Bunch 67 were the best of the U.S. entries, although RMP 12 yielded the highest. Based on numerical yields, RMP 12 seemed to be the most stable in yield, as evidenced by having the highest average yield over the three year period.

Because rosette is endemic at Niangoloko, and peanut rust is variable from year to year, variety trials are highly variable. RMP 12 is rosette-resistant but highly susceptible to rust. Nematodes are also thought to be important in the area. No entry was found to compete with RMP 12 when rosette was important, as in 1986, and to a lesser extent in 1985. Interestingly, in 1987, two U.S. cultivars, Early Runner and Tamnut 74 produced higher pod yields than RMP 12, which had an uncharacteristically low yield. Because rust did not appear to be more severe than in previous years, other factors might have contributed to its relatively low yield. Unless rosette can be controlled by other means, or rust and rosette resistant cultivars can be developed, RMP 12 will remain the most reliable variety for this area of Burkina Faso.

TS 32-1 ranked no higher than seventh in seed yield and fifth in pod yield in any of the three years at Gampela. No single variety stood out as superior in all three years, however. Florunner, Pronto, Sunrunner, Va Bunch 67, and New Mexico Valencia C showed the most promise over the three years and should be continued in further tests.

TS 32-1 performed slightly better than the ten U.S. varieties at Saria ranking fourth in seed yield in 1987. Pod yields of Va Bunch 67, Tamnut 74, and Spanco were numerically higher than TS32-1 in all three years. None of these three entries produced higher seed yields than the check in all three years, although Va Bunch 67 did yield the most seed in 1985 and 1987. Florunner and Sunrunner yielded more seed than TS 32-1 in all three years, being the top two entries in 1986, and exceeded only by Va Bunch 67 in 1987. Based on these data, Va Bunch 67, Florunner, Sunrunner, and Tamnut 74 should be tested further.

Results from Tenkodogo showed a clear superiority of Sunrunner, Florunner, and Va Bunch 67 over TS 32-1, in all years for both pod and seed yield. Early Runner did well in 1985 and 1987, but had extremely poor yields in 1986.

Given the relatively short growing season and low rainfall typical of Saria, Gampela, and Tenkodogo, it was surprising to find the virginia and runner varieties performed so well over years and locations, as compared to TS 32-1. Differential compensation and plant stands might have biased the results as reported. While the varieties mentioned should continue to be tested at the respective locations, at some time it would be desirable to compare performance of these lines with TS 32-1 under conventional production practices.

## 7.2 Disease Resistance

### 7.2.1 Burkina Faso Evaluations

#### 7.2.1.1 General

Observations were made in Southwestern Burkina Faso at Bobo-Dioulasso and Niangoloko for reaction to leafspot, rust, pod disease, and insects. These locations, with average annual rainfalls of 1000-1200mm, typify the long-season peanut growing region of Burkina Faso and other areas in West Africa. Entries tested included both spanish and runner breeding lines that showed both rust and



leafspot resistance in preliminary tests in the U.S. RMP 12 was included as a West African check variety, and Southern Runner as a U.S. check. The thirty-six entries were planted in randomized complete blocks with two replications. Each entry was planted in two-row plots with plants spaced at 15cm in rows 40cm apart. Planting and harvest dates were June 30 and October 12 at Bobo-Dioulasso and July 4 and October 20 at Niangoloko.

Rust and leafspot assessments were made at approximately two week intervals beginning when disease symptoms were first observed. Pod disease and insect damage was assessed at 51 DAP, 86 DAP, and harvest at Bobo-Dioulasso, and at 57 DAP, 88 DAP, and harvest at Niangoloko. Rust and leafspot were scored using the ICRISAT scale (Peanut Science 9:6-10). Total pods and pods with disease and or sign of insect damage were counted.

#### 7.2.1.2 Leafspot

Leafspot was apparent in plots at Bobo-Dioulasso more than five weeks earlier than in plots at Niangoloko. By harvest, however, mean infection over entries was comparable at the two locations. None of the twenty-six entries being evaluated for the first time were as resistant as RMP 12 at either location. Four of nine entries tested at Niangoloko in 1986, and at both locations in 1987 had slightly lower ICRISAT scores (in parentheses) than RMP 12 (5.0) averaged over the three environments: US 689 (3.7); Tx855101 (4.3); US 482 (4.4); and Tx855157 (4.4).

#### 7.2.1.3 Rust

In 1987, rust was first observed at Niangoloko the first week in September; no rust was observed in Bobo-Dioulasso. At 108 DAP, virtually all entries had lower rust scores than RMP 12, which is highly susceptible to rust. Among entries being grown for the first time, the average scores for CES-14R, CES-14T, CES-58, and TP158-4A-BP were less than 4.0, compared to 8.0 for RMP 12. Five entries grown in both 1986 and 1987 at Niangoloko were highly resistant, scoring three or less in both years. Among these five lines were Tx855157 and Tx855101, two pod rot resistant selections derived from a cross of TxAG-3 and Tamnut 74.

#### 7.2.1.4 Pod Disease and Insect Damage

Pod disease and insect damage were low at both locations. *Sclerotium rolfsii*, *Pythium*, and *Rhizoctonia* were most commonly observed. Among the first-year entries, CES-3 had a slightly higher incidence of pod disease than many entries at both locations, averaging 11.2% of pods diseased.

### 7.2.2 Yoakum, Texas Evaluations

#### 7.2.2.1 General

Peanut germplasm from Central and South America, Africa, India, and the U.S. was evaluated in several field tests for disease resistance. Entries were screened for resistance to early leafspot caused by *Cercospora arachidicola*, late leafspot caused by *Cercosporidium personatum*, and pod disease caused by *S. rolfsii* and *Rhizoctonia solani*. Southern blight infection was determined by counting the number of target sites (disease loci) per experimental plot. Pod disease was determined visually using a 0 to 10 scale where 0=no disease and 10=completely diseased. Multiplied by ten, the scores approximate the percentage of pod discoloration resulting from disease. Leafspot disease was assessed with either the nine point ICRISAT scale for late leafspot, the 10-point Florida scale, or the Boyle and Jensen Infection-Defoliation method (BJID). The latter method involves selecting from three to five mainstems from each plot and counting the total number of leaflets, the number of leaflets infected with one or more spots, and the number of defoliated leaflets per mainstem. Some tests were scored by more than one method.

#### 7.2.2.2 ICRISAT Groundnut Foliar Disease Nursery

Forty peanut entries from ICRISAT and four U.S. cultivars (Southern Runner, Florunner, Tamnut 74, and Starr) grown without fungicide were evaluated in a completely randomized design with four replications. Each plot consisted of two 8.5m rows, 0.9m apart. Entries were planted July 1 and

harvested according to maturity at 106, 121, or 126 days after planting (DAP). Each plot was inoculated with 55g of *S. rolfisii* inoculum at 54 DAP.

*C. arachidicola* was the predominant pathogen early in the season, but *Puccinia arachidis* (not evaluated) and *C. personatum* prevailed later. ICRISAT leafspot scores ranged from 1.7-3.2, 3.0-5.7, and 4.7-8.2 at 51, 82, and 96 DAP, respectively. At 82 DAP, two entries, RMP 12 and PI393527-B, had significantly lower disease scores than the most resistant U.S. cultivar, Southern Runner. At 96 DAP, over half the ICRISAT entries were rated significantly more resistant than Southern Runner.

Five of forty ICRISAT lines (NcAc17135, NcAc17127, NcAc171324, NcAc17132, and PI390593) scored significantly lower in %infection + %defoliation than Southern Runner at 98DAP. Southern Runner averaged 44% infection and 42% defoliation for a total of 86% and was the lowest in score of U.S. checks. The five ICRISAT lines ranged from 73-77% in BJID score; all were significantly more resistant than Southern Runner when evaluated with the ICRISAT scale at 96 DAP, and when only % defoliation was evaluated. One of the five entries, PI390593, was the least defoliated (19%).

No entries had significantly fewer Southern blight target sites than the U.S. cultivars evaluated. Scores, determined at harvest, ranged from 0 to 10.5. Three entries, NcAc17132, NcAc17135, and PI393526 had no infection.

Overall pod disease was very low and ranged from 0.3 to 1.8. No ICRISAT lines had scores significantly lower than the U.S. cultivars.

PI390593 was the highest yielding ICRISAT line (3310 kg/ha, 69% TK). It yielded significantly more than Florunner (2688 kg/ha, 80% TK) and Starr (2435 kg/ha, 76% TK) but not significantly more than Southern Runner (3017 kg/ha 79% TK), and Tamnut 74 (2887 kg/ha, 75% TK). Six other ICRISAT lines were not significantly different than PI390593, yields ranging from 2862 - 2724 kg/ha.

### 7.2.2.3 RCM Lines Observation Test

Two hundred fifty *A. hypogaea* entries from South America were evaluated for leafspot reaction 85, 106, and 116 DAP using the 10-point Florida scale, and for pod rot at harvest using the 1 - 10 scale. Test entries were unreplicated and planted in single 4m rows. Twelve single row plots each of Tamnut 74 and Southern Runner, and twenty-three single row plots of Florunner were dispersed among the test entries as checks.

Four entries, RCM753, RCM754, RCM755, and RCM757 had the lowest leafspot scores at all three rating dates (3,5,5 at 85, 106, 116 DAP, respectively) in contrast to the most resistant check, Southern Runner, which averaged 3.9, 5.8, and 6.3 at the three respective rating dates. These entries will be evaluated again during the next growing season. None of the two hundred fifty lines was notable for pod rot reaction; a few were comparable to the lowest plot of the Southern Runner entries, which was the lowest of the three checks (1.9 +/- 0.5)

## 7.2.3 Breeding Program for Leafspot Resistance

### 7.2.3.1 Backcross Program

Field evaluations were made of progenies from the program designed to transfer leafspot resistance from three wild species of *Arachis* into cultivated peanut lines. Of sixty-four lines evaluated, twenty-five were rated better than the susceptible recurrent parents in yield, grade, 100-seed weight, maturity, or leafspot resistance. Some of the lines were superior in more than one character and several lines were better in all characters. None of the lines were immune to early or late leafspot. This first field evaluation of the lines scored as resistant in laboratory tests using detached leaves indicated that the "resistant" lines were not carrying the level of field resistance needed to develop resistant cultivars. Therefore, the program has been regressed to an earlier backcross generation to obtain more genes for leafspot resistance in order to obtain a higher level of field resistance. The selection process will be modified so that subsequent backcrosses will be made on backcross F<sub>2</sub> plants which test resistant,

rather than backcross  $F_1$ 's which test as resistant. This should enhance the probability of recovering resistant alleles at more of the loci and give higher levels of field resistance to leafspot.

In order to utilize those progeny already developed, and to gain information on how to handle future populations of similar origin, the  $F_3$  progenies from the highest yielding, most leafspot resistant lines will be evaluated in replicated yield trials. This should indicate if useful genes for yield have been introduced into the introgression hybrids.

### 7.2.3.2 ETC Leafspot Resistance Selection Program

$F_4$  progenies from crosses of three amphiploid selections and Florunner, Tamnut 74, Sn73-30, TP107-3-8, Va72R, and New Mexico Valencia A were grown for evaluation and selection. In 1985 and 1986, plants were selected from each population on the basis of low %infection, low %defoliation, and high pod load. Plant rows and bulk populations comprised of selected plants will be evaluated at Yoakum for leafspot resistance and agronomic acceptability.

### 7.2.4 Breeding for Multiple Adversity Tolerance

The final crosses in the convergent crossing program were accomplished. As reported in previous years, this program was designed to attempt to combine several desirable characters of yield, disease resistance, drought tolerance, and earliness into one group of lines.

## 7.3 Pod Rot Resistant Selections Evaluation

### 7.3.1 Pod Rot #1

Five selections derived from a cross of TxAG-3 and Florunner, and six lines derived from crosses of Tamnut 74 with either TxAG-3 or US 224 were evaluated for yield, quality, and pod disease under irrigated conditions at Yoakum and Bryan, Texas, in four replications using a randomized complete block design. TxAG-3, Tamrun 88, and Florunner were included as checks. *Pythium myriotylum*, *Rhizoctonia solani*, and *Sclerotium rolfsii* were the primary pathogens detected. Plots were planted May 22 and June 24 at Bryan and Yoakum, respectively, and harvested at maturity.

BRYAN					YOAKUM				
Entry	Pods (kg/ha)	SMK %	TK %	Pod Rot %	Entry	Pods (kg/ha)	SMK %	TK %	Pod Rot %
Tx855108	4404 a	72 ab	79 ab	13.3 b-e	Tx855206	4291 a	69 e-g	76 d-f	6.7 c
Tx855113	4252 ab	66 de	76 c	18.3 ab	Tx855229	3988 ab	67 g	78 c-e	7.0 c
Tx855155	4182 ab	70 a-d	79 ab	11.5 c-f	TxAG-3	3672 a-e	69 fg	74 f	9.0 bc
Tamrun 88	4022 a-c	69 a-d	79 ab	22.5 a	Tamrun 88	3090 b-e	76 ab	81 ab	9.0 bc
Florunner	3770 b-e	71 a-c	78 a-c	8.0 d-g	Florunner	3045 c-e	71 d-g	79 b-d	15.8 a
TxAG-3	2383 gh	63 e	68 fg	6.8 fg					

Weight of 100 seed was higher for entries grown in Yoakum, ranging from 44g to 65g at Bryan, and from 52g to 69g at Yoakum. At Bryan, Tx855106 yielded significantly more pods and had a significantly higher value per acre than did Florunner, but was not significantly greater than Tamrun 88 for either parameter. No selection statistically exceeded Florunner in %SMK or %TK. Only the top and bottom ranked entries differed significantly at Yoakum in value per acre. Tx855106 ranked twelfth and thirteenth in value per acre and yield, respectively, and did not differ significantly from Florunner or Tamrun 88. No entries at Yoakum were significantly better than Tamrun 88 in either %TK or %SMK. Tx835841 and TxAG-3 exceeded all other entries in 100-seed weight at both locations.

At Yoakum, pod rot infection ranged from 6.8% to 15.8%, Florunner having the most disease. Twelve of fourteen entries were not statistically different from the pod rot resistant parent TxAG-3, which had a 9.0% score. Scores at Bryan covered a slightly wider range. Three selections did not differ significantly from Tamrun 88, which had the most infection (22.5%). Four selections and Florunner were not significantly different from TxAG-3.

### 7.3.2 Pod Rot #2

Eight selections from crosses between Tamnut 74 and TxAG-3 were compared to checks at both Bryan and Yoakum in a randomized complete block design with four replications. Florunner, TxAG-3, Tamnut 74 and Toalson were checks at both locations. Plots were planted May 21 at Bryan and June 24 at Yoakum and harvested at maturity.

BRYAN					YOAKUM				
Entry	Pods (kg/ha)	SMK %	TK %	Pod Rot %	Entry	Pods (kg/ha)	SMK %	TK %	Pod Rot %
Tx855158	4378 a	68 a-c	74 b-d	6.0 h	Tx855139	3588 a	70 bc	75 d	9.0 d
Tx855146	4162 ab	65 c-e	71 de	5.8 h	TxAG-3	3542 a	71 b	75 d	11.0 cd
Florunner	3986 a-c	64 c-e	76 b	19.5 bc	Tx855122	3407 a	77 a	82 a	14.8 bc
Tx855122	3867 a-c	72 a	79 a	14.5 c-e	Tx855146	3400 a	67 de	73 fg	8.3 d
TxAG-3	2789 ef	61 de	67 g	9.0 f-h	Florunner	3237 a	71 bc	79 b	21.8 a
Tamnut 74	261b fg	61 de	72 c c	26.0 a	Tamnut 74	3046 a	70 bc	76 d	17.7 ab

In the Bryan test, no entries significantly exceeded Florunner in either yield or value per acre. Tx855158 was numerically highest for both parameters, followed by Tx855146 in yield and Tx855122 in value per acre. Tx855122 ranked highest for both %SMK and %TK and was significantly greater than Florunner in both characteristics. Tx855122 was very close to Florunner in 100-seed weight, and both entries were significantly higher than other selections. TxAG-3 had the highest 100-seed weight. Tx855122 was significantly greater than all other entries for both %TK and %SMK. No significant differences among entries were detected for pod yield, and only the top and bottom ranked entries differed significantly in value per acre; however, Tx855122 was ranked highest for value, and third for yield. Entries appeared more mature at Yoakum. As in the Pod Rot 1 test, 100-seed weights were higher at Yoakum than at Bryan, ranging from 40g to 70g at the former location, and from 36g to 61g at the latter location. As at Bryan, TxAG-3 had the highest 100-seed weight. Tx855122 was the third highest, and was significantly greater than all other entries except TxAG-3 and Florunner.

Pod rot varied from 5.3% to 26% among entries at Bryan, and from 8.3% to 21.8% among Yoakum entries. Seven of eight selections were not significantly different from TxAG-3 in pod rot infection, although all checks did have more infection. Tx855122, the entry with the highest %SMK, scored higher for pod rot than TxAG-3. No selection differed significantly from TxAG-3 at Yoakum.

## 7.4 Drought Stress Tolerance

### 7.4.1 Senegal Yield Tests

#### 7.4.1.1 Bambey

Peanut clump was very severe, with up to 60% of plants in a plot affected. Foliar pathogens and aphids were not of major importance. Abnormally high soil pH in the experimental field was discovered after planting, evidenced by numerous areas of yellowing plants. The surface pH was found to be 8, while at 50cm, the pH was 9. 200kg/ha of ammonium sulphate was applied three times at one week intervals (600 kg/ha total) to lower the pH.

In four tests in which the three West African cultivars 57-422, 55-437, and 73-30 were all grown, 57-422 yielded an average of 1745 kg/ha, 280 kg/ha more than 73-30 and 300 kg/ha more than 55-437. Comparing yields of 57-422 with 55-437 over 12 years, the former variety was superior except when the rainy season was delayed. 55-437 was superior in shorter growing seasons.

A ten variety yield test conducted in a balanced incomplete block design with six replications compared peanut varieties from six countries with three West African cultivars.

Entry	Origin	Pods (kg/ha)	Vines (kg/ha)	TK %	Good 100		Cycle (days)	Peanut Clump %
					Seed %	Seed g		
PI 1174	China	1840	3540	70	57	54	85	11
Spanco	U.S.	1665	3105	72	59	34	81	17
796A	USSR	1625	3010	74	61	40	85	24
TG 3	India	1520	3400	70	58	48	85	20
TS 32-1	W. Africa	1460	2695	70	56	43	85	33
Tatui	Brazil	1360	3220	70	56	50	85	19
Pronto	U.S.	1320	2790	72	59	35	79	28
H Sefa	Phillipines	1250	2890	66	56	42	85	34
73-30	W. Africa	1250	2355	71	59	37	90	21
55-437	W. Africa	1055	2420	71	55	34	85	38

1987 yield results were confounded to some degree with peanut clump infection; the varieties with lower percentages of infection generally had higher pod and haulm yields. PI 1174 had the highest pod and haulm yields, 10.5% and 4.1%, respectively, more than the second best entries. All entries ranked higher in terms of pod and haulm yield than the two local varieties 55-437 and 73-30. PI 1174 also had large seeds, as indicated by 100-seed weight. Of interest was the earliness exhibited by Pronto and Spanco compared to 55-437; 6 and 4 days earlier, respectively. PI 1174 exceeded local entries in pod yield by almost 20% based on three years of results. Vine yields and % good seeds were approximately the same. PI 1174 has been included in a 1988 yield test in Bryan, Texas to assess its performance, and has been recommended for multiple location testing in Senegal.

Fourteen U.S. breeding lines, three U.S. varieties (Toalson, Starr, Langley), and three West African cultivars (55-437, 73-30, 73-33) were evaluated in three replications of a 4 x 5 rectangular lattice design.

Entry	Pods (kg/ha)	Vines (kg/ha)	TK %	Good 100		Cycle (days)	Peanut Clump %
				Seed %	Seed (g)		
Starr	2105	2910	75	59	38	85	13
Toalson	2050	3725	71	59	42	91	2
Tx798736	1980	3030	73	59	38	88	22
Tx782354	1605	3035	75	59	36	97	22
Tx782447	1560	2690	74	60	37	91	21
55-437	1500	3105	74	59	36	85	14

Pod yields were near normal despite the high incidence of clump. Starr had the highest yield, 2105 kg/ha, compared to the highest yielding local cultivar, 55-437, which yielded 1500 kg/ha. With one exception, all U.S. entries had higher average yields than the local checks. In general, however, shelling percentages were lower for the U.S. entries than for the two local checks. Based on these data, and on multi-year data, the five entries in the above table will be continued in further performance testing in Senegal.

#### 7.4.1.2 Nioro

Four U.S. breeding lines, one U.S. cultivar (Langley), and the local check 73-33 were compared in six replications of a randomized complete block design.

Entry	Pods (kg/ha)	Vines (kg/ha)	TK %	Good 100		Cycle (days)	Leafspot (ICRISAT)			
				Seed %	Seed (g)		-----DAP----- 60 75 90 105			
73-33	3325	4085	73	62	53	103	2.5	5.0	7.0	8.0
TP 107-11-4-15	3125	3250	78	70	60	100	3.5	4.2	7.0	9.0
Langley	3035	4425	75	63	60	103	3.2	6.0	8.0	9.0
Tx813929	2935	3675	74	65	63	108	3.3	4.2	6.0	7.0
Tx811923	2875	4100	74	69	37	103	3.7	6.0	8.0	9.0
Tx762431	2820	4455	75	68	38	103	3.0	6.0	8.0	9.0

Leafspot appeared early and spread quickly. Tx813929 appeared least susceptible throughout the growing season. Two entries, Tx762431 and Langley, yielded approximately 350 kg/ha more hay than the local check, 73-33; however, no entry exceeded 73-33 in pod yield. In this test, 73-33 had the lowest shelling percentages. Based on four years of tests, however, no entries exceeded 73-33 or 28-206 in pod yield.

In a second test involving selections from a cross of 57-422 with Samaru 1064, yields of most entries exceeded 3000 kg/ha. While no entries numerically exceeded the 3575 kg/ha yield of the check 73-33, only three of twenty-three selections yielded less than 28-206. Eight selections yielded over 3400 kg/ha of pods.

#### 7.4.2 Texas Dryland Yield Test

Twelve ICRISAT lines reported to be drought tolerant, two Senegalese varieties 55-437, 59-127), and two early U.S. cultivars (Starr, Pronto) were evaluated in four replications of a randomized complete block design. Planting and harvest dates were June 26 and October 1, respectively. Each plot consisted of two 4.6m rows spaced 1m apart.

Entry	Pods (kg/ha)	SMK %	TK %	Value (\$/ha)
J-11/Robut 33-1	2422 a	58 b-d	71 de	1356 a
Ah7807	2039 b	66 a	76 a	1298 ab
Ec109271	1995 b	60 bc	74 a-c	1174 a-c
55-437	1978 b	59 bc	73 b-d	1160 bc
Pronto	1981 b	62 ab	75 ab	1194 a-c
Starr	1558 de	56 cd	74 a-c	865 e-g

J-11/Robut 33-1 yielded significantly more pods than any other test entry. In spite of lower %SMK and %TK scores, it ranked highest in value per acre (based on 1987 USDA loan rates). Ah7807 also yielded well; while not significantly better in pod yield than Pronto or 55-437, its high shelling percentage ranked it above Pronto in value per hectare. Ec109271 also was not significantly different from Pronto in value/ha.

Over two years, J-11/Robut33-1 averaged 113% of Pronto for pod yield, and 109% of Pronto for value. Ah7807 was the only other entry superior in yield to Pronto over the two years, averaging 106% and 112% of Pronto for pod yield and value, respectively.

#### 7.4.3 Yoakum, Texas Drought Stress Research

##### 7.4.3.1 Yield Studies

The 1987 growing season was characterized by a dry spring, followed by a period of continuous rainfall in June, mid-summer and early-fall drought, and nearly normal rainfall in November. Monthly rainfall amounts for 1985 - 1987 and over the last thirty years are provided below:

Month	1985	1986	1987	30-yr Mean
January	71	28	41	61
February	81	36	121	65
March	83	35	17	50
April	162	14	8	82
May	84	133	123	108
June	113	193	402	83
July	71	3	55	67
August	7	91	35	74
September	93	133	27	109
October	93	113	20	76
November	233	59	90	65
December	30	116	98	62
TOTAL	1120	952	1137	902

The heavy June rainfall delayed planting until June 24, later than was desired. Late planting sometimes causes the major pod-filling and maturing time to occur in wet fall periods, but in 1987 there was drought pressure throughout much of the growing season.

Selected cultivars from West Africa and the U.S. have been compared under rainfed conditions for up to four years at the Texas Agricultural Experiment Station at Yoakum. The following table shows yield, grade (percent sound mature kernels + sound splits) and other kernels (O.K.; percent sound, undersized kernels) for the 1987 crop year, while the next table provides similar data for one to four year period (1984-1987):

Entry	Yield (kg/ha)	Grade %	OK %
Langley	1904 a	61.0 bc	11.8 ab
Starr	1715 ab	63.5 ab	8.3 cd
Pronto	1574 abc	67.3 a	6.5 d
Florunner	1573 abc	58.0 c	13.8 a
Tamnut-74	1409 bc	58.0 c	11.3 abc
SN 73-33	1315 cd	56.8 c	13.3 a
SN 55-437	1268 cd	59.3 bc	11.3 abc
SN 57-422	1016 de	64.0 ab	7.8 d
SN 73-30	908 e	60.3 bc	9.5 bcd
SN 28-206	746 e	57.8 c	8.0 d

Entry	Number of Years Tested	Yield (kg/ha)	Grade %	O.K. %
Florunner	4	1844	60	14
Langley	4	1809	60	16
Pronto	4	1609	66	8
SN 73-33	4	1534	55	16
SN 73-30	4	1337	60	11
Starr	4	1221	54	16
Early Bunch	3	1312	38	36
SN 59-127	3	1267	54	13
SN 55-437	1	1268	59	11
SN 57-422	1	1016	64	8
SN 28-206	1	746	58	8

Under the rainfall patterns at Yoakum, many of the U.S. cultivars which were selected for performance under adequate water have equalled or exceeded the West African lines which were selected for drought resistance. Throughout the research SN73-33 from Senegal has been among the most productive spanish entries.

#### 7.4.3.2 End-of-Row Effects

One aspect of the research was to compare plant characteristics from the middle of the plot rows with those of the three end plants adjacent to the alleys. The end plants are subjected to less competition for water than are the middle plants, because they are able to draw reserves from the fallow alley. Typically, the plants near the ends are larger and have more peanuts. It has been reported in some crop species, that more drought resistant lines respond less to this greater water availability than do drought susceptible lines. In 1985, 1986, and 1987, we compared the end three plants to three plants in the middle in each plot. Data collected included number of pods per plant and air dry pod weight per plant; percent of pods which were empty (pops), percent single-kernel pods, and percent double-kernel pods; and plant height and width. Differences were observed among entries for end values, for mid-plot values, and for end vs. mid-plot values for some variables. There were, however, few significant entry X position interactions and these were not consistent throughout the three-year period. There were a few interesting observations which may prove useful in subsequent research. In 1986, there were significant entry X position interactions in percent pops and percent double-kernel pods. In both interactions, only Pronto, Tamnut 74, SN73-33, and SN59-127

had significant differences between end and middle values--Pronto and Tamnut 74 had higher percent pops and lower percent double-kernel pods on the end than on the middle plants. Conversely, SN73-33 and SN59-127 had lower percent pops and higher percent double-kernel pods on the end than on the middle plants. In 1987, there was a significant entry X position interaction for double-kernel pods. But in this instance, all entries had higher means for the end than the middle plants, except Starr and SN55-437, for which the end and middle plant values were equal.

#### 7.4.3.3 Leaf Relative Water Content

In 1987, we began collecting data on Leaf Relative Water Content (RWC), which compares the amount of water the leaf contains under stress with the amount of water the leaf is capable of holding. RWC values were calculated as follows:

$$\text{RWC} = ((\text{FRESH WT}-\text{DRY WT})/(\text{SATURATED WT}-\text{DRY WT})) \times 100$$

Five leaves were collected randomly within each plot at dawn and at solar noon at 50, 60, 70, 80, and 100 days after planting (DAP) for RWC measurements. One leaflet from each leaf was combined with other leaflets from that plot and sealed in a preweighed glass vial. Fresh weights were taken and the leaflets then saturated with distilled water overnight. Saturated weights were taken, leaflets dried to a constant weight in an oven at 80C, and dry weight determined.

The following tables show mean RWC values at dawn and afternoon for each collection date and the season average for the peanut entries.

****MORNING COLLECTION****						
Plant Age in Days After Planting						
ENTRY	50	60	70	80	100	MEAN
SN 73-30	96.4 ab	92.9 abc	96.8 a	95.4 a	95.3 ab	95.4 a
Pronto	96.7 a	93.7 ab	94.8 abc	95.2 ab	97.0 a	95.2 a
SN 28-206	95.8 abc	94.3 ab	96.2 ab	95.0 abc	94.3 ab	95.1 a
SN 55-437	94.3 bc	95.4 a	93.9 bc	94.5 abcd	96.0 a	94.8 a
SN 73-33	93.9 c	90.8 cd	94.5 abc	94.0 abcd	95.0 ab	93.7 b
Starr	94.3 bc	92.5 bc	92.9 c	93.9 abcd	95.0 ab	93.6 b
Florunner	93.8 c	92.0 bcd	93.6 bc	93.4 bcd	94.3 ab	93.4 b
Tamnut 74	93.6 c	92.1 bc	92.6 c	93.2 cd	94.8 ab	93.3 b
Langley	93.8 c	90.8 cd	93.6 bc	92.7 d	92.8 b	92.7 b
SN 57-422	91.3 d	89.5 d	92.5 c	93.5 bcd	93.0 b	92.0 c

****AFTERNOON COLLECTION****						
Plant Age in Days After Planting						
ENTRY	50	60	70	80	100	MEAN
SN 73-30	85.1 a	78.1 a	86.9 a	78.8 a	84.0 ab	82.6 a
Pronto	78.6 ab	67.5 bc	82.1 b	75.2 ab	82.5 ab	77.2 b
SN 28-206	77.2 ab	68.4 bc	84.0 ab	75.4 ab	79.8 ab	77.0 b
SN 55-437	79.0 ab	66.6 bc	82.5 b	75.9 ab	83.0 ab	77.6 b
SN 73-33	76.6 ab	67.3 bc	82.9 b	73.2 b	79.0 ab	75.8 bc
Starr	76.3 ab	67.4 bc	83.2 b	74.6 ab	95.0 ab	76.7 b
Florunner	76.5 ab	65.5 cd	83.4 b	75.0 ab	74.5 b	75.0 bc
Tamnut 74	72.5 b	66.7 bc	84.2 ab	76.1 ab	86.7 a	77.6 b
Langley	72.0 b	62.6 d	83.4 b	73.9 b	74.0 b	73.2 c
SN 57-422	77.1 ab	69.8 b	81.8 b	74.1 b	76.0 ab	75.7 bc

At both collection times, there were significant differences among entries, with SN73-30 the highest or in the highest Duncan's Multiple Range grouping in each comparison. Under 1987 conditions, the



dawn collection seemed to do the best job in separating out entries, but the groupings were not obviously related to yield performance under water stress.

Hydraulic press readings were made on other leaflets of the same leaves collected for RWC determinations to determine if this rapid indicator of water potential might have merit in selection for drought resistance in peanut. Data are not presented in this report; as in the case of RWC, however, there were differences among entries, but without obvious relationship to drought resistance. Both variables are being studied in 1988.

## 7.5 Earliness

### 7.5.1 Yield Evaluations of Early Maturing Lines

Thirteen early ICRISAT lines and other early germplasm were evaluated for yield and quality in a randomized complete block design with four replications. Plots consisted of two 4.6m rows spaced 0.9m apart. Planting and harvesting dates were June 26 and October 1, respectively.

Five early ICRISAT lines and Langley yielded significantly more pods than the highest U.S. cultivar, Starr. Three of the five also exceeded Starr in value. Starr and 55-437 performed very similarly in both yield and value. One entry, ICGS(E)-56, was ranked highest in pod yield in both years, and in value in 1986. It also has performed relatively well in an ICRISAT test in Niger. Langley, an early runner cultivar, ranked second in yield in both years, but was poorly filled in 1987. Over both years of testing, seven entries exceeded both Starr and 55-437 in yield, and six of the seven exceeded the checks in value. ICGS(E)-56 averaged 132% and 128% of Starr for pod yield and value, respectively, and performed best over the two years.

### 7.5.2 Selection for Earliness

#### 7.5.2.1 SEM Test

In 1985,  $F_2$  plants from a cross of TxAg-1 x Chico and its reciprocal cross were space-planted for evaluation of pod load, agronomic characteristics, and earliness. Selected plants and parents were evaluated in 1986 as plant rows. Evaluations of selected  $F_4$  lines were made in two preliminary irrigated yield tests. Planting and harvest dates were June 16 and September 30. In one test, nineteen lines, Starr, 55-437 (an early Senegal variety), and TxAG-1 were compared in three replications of a randomized complete block design. Yields ranged from 3098 to 2269 kg/ha. Only two lines exceeded the highest check, Starr, but the difference was not significant. Interestingly, all lines had numerically higher total kernel percentages (%TK) than any of the three checks; fourteen of nineteen were significantly greater, and the highest line, B86608 (78%) was the second highest in pod yield.

In the second test, thirteen  $F_4$  lines were compared to Pronto, Starr, and 55-437 in two replications of a randomized complete block design. No significant differences were found in pod yield. Yields ranged from 2809 to 1920 kg/ha. Pronto ranked highest. As in the three replicate test, most of the top ranking entries for %TK were  $F_4$  lines. Nine lines ranked higher than the top check, Pronto, although none were significantly greater.

#### 7.5.2.2 Multiple Harvest Date Test

The effect of selection for earliness by varying harvest date is being studied in six populations composed of Sn55-437 x Chico, TxAg-1 x Chico, Sn55-437 x TxAg-1, and each of the reciprocal crosses.  $F_3$  and  $F_4$  bulks of each of the six populations were planted in 1986. At 79 (H1) and 90 (H2) DAP, ten plants per population were selected based on pod load, size, and distribution. The proportion of mature pods by plant was determined by the hull scrape method. A third harvest (H3) at 103 DAP was made with no selection for earliness. The three subpopulations formed by harvest dates within each of the six main populations were evaluated for yield in Maradi, Niger and Nioro, Senegal, and for yield and maturity at Bryan, Texas to determine if selection was effective.

Preliminary analysis of the Bryan test indicated the H1 populations significantly exceeded ( $p=0.10$ ) their H3 counterparts for percent mature pods in three of six populations, and numerically exceeded

the H3 in two others. Within the H1 class, TxAG-1/Sn55-437 ranked highest in percent of mature pods, differing by 10% from the second highest entry. Analysis of H2 vs H3 populations were not yet available.

In Senegal, the three harvest subpopulations of each of the six crosses were compared with the local check, 55-437, which was a component of four of the populations. The check had a higher percent of mature pods than any of the three harvest classes combined over populations (75% for check vs. 57%, 57%, and 68% for H1, H2, and H3, respectively). The check was also consistently higher than any of the harvest populations for pod weight per plant, shelling percentage, and 100-seed weight.

Poor rainfall in Niger severely depressed yields. H1 and H2 exceeded H3 in both pod and seed yield as shown:

Sub-Population	Pods (kg/ha)	Seeds (kg/ha)
H1	411	189
H2	450	213
H3	355	159

In the cross Sn55-437/Chico and its reciprocal the yield of H1 lines exceeded that of H2 line. In the other four crosses, however, the reverse was true.

### 7.5.3 Inheritance of Earliness

Five early maturing cultivars (Chico, Sn55-437, TxAG-1, TxAG-2, and Tx851856) were crossed in a complete diallel, and parental, F<sub>1</sub>, and F<sub>2</sub> generations studied in 1987 in four replications. Flowering pattern (days to first, fifth, etc. flower) and pod development at harvest were evaluated.

Differences among parents, and among progenies within a cross were found for rate of emergence, flowering pattern, and numbers of full size and mature pods. Transgressive segregates earlier in flower setting and number of both full and mature pods were identified. The low correlations between pod parameters and flowering pattern indicated flowering pattern, particularly days to first flower, could not be effectively used to measure earliness in the early-maturing germplasm evaluated. The number of full-size pods and number of mature pods, combined with early harvest appeared to be better criteria for selecting early germplasm.

Broad-sense heritability estimates varied widely among crosses and over parameters measured. Chico/Tx851856 and its reciprocal had the highest heritability for both flowering pattern and number of full or mature pods, while crosses of TxAG-1 with Chico were among the lowest. No clearly discernable differences were detected in segregation pattern in any of the crosses for pod number parameters, and therefore numbers of genes involved in the control of the parameters were not determined. No significant differences in general or specific combining ability were found for parents or single crosses.

## 7.6 Evaluation of Resistance to Tomato Spotted Wilt Virus

### 7.6.1 General

In five replicated tests ninety-three varieties and germplasm lines were evaluated for reaction to Tomato Spotted Wilt Virus (TWSV) under natural infection conditions at Pearsall in Frio County, Tx. In addition to adapted U.S. breeding lines and cultivars, entries included sixteen breeding lines from the North Carolina State Experiment Station, twenty-nine cultivars and breeding lines from ICRISAT, and ten lines from the Florida breeding program. Many entries had been evaluated previously in 1986. Yield and grade were also measured. Entries in a test were replicated four times in randomized complete block or split plot designs. Entries were dug according to maturity at 128, 143, or 149 DAP. The number of row feet with spotted wilt (SW) disease symptoms were counted four or five times during the growing season. Disease scores were converted into "area under disease progress

curve" (AUDPC) scores which consider both level and time of infection. Early infection and high disease incidence contribute to high scores. Disease pressure was less in 1987 than in 1986.

Among U.S. runner varieties, GK-7 generally had the lowest disease score and highest pod yield compared to Florunner and Tamrun 88. The latter variety had the most disease among these entries. Southern Runner also had low disease scores. Tx798736, and a reselection, Tx798736-1, were among the highest yielding and lowest in disease score among early maturing spanish entries. This breeding line also has demonstrated low pod rot and *Sclerotinia* blight disease reactions.

Several lines from North Carolina exceeded the Florunner and Southern Runner checks in yield or disease score. One entry, designated NC343/GK53-17, a virginia market type, had both the highest pod yield in the test (115% of Florunner) and lowest disease score among North Carolina entries (33% of Florunner). This entry had a higher disease score than Florunner in 1986, however.

Among the ICRISAT lines being tested for the first time, two lines, Ah7729 and 55-B3-16, virginia and runner market types, respectively, had both high pod yields and low disease scores. The yield of 55-B3-16 was 110% of Florunner, and disease score of both entries was only 17% of Florunner. Several ICRISAT lines being evaluated for SW reaction a second time had higher yields than either Florunner or Southern Runner while having nearly equivalent disease scores. GBPRS 302, a runner market type, was 122% of Florunner's pod yield. It also had high pod yield low disease score in 1986.

While none of the Florida breeding lines had lower disease scores than Southern Runner, UF81206-2, a runner market type, yielded 108% of Southern Runner, the highest yielding check in the test.

## 7.7 Disease Surveys

### 7.7.1 Burkina Faso

A disease survey was conducted during September, 1987 to assess the relative importance of various groundnut diseases in the major groundnut production areas of Burkina Faso. The survey was jointly sponsored by ICRISAT, IRHO, Peanut CRSP, and the University of Ouagadougou. Sixty-four fields were examined in the eastern, central, south-central, and southern provinces of the country.

Early leafspot was severe in nearly all fields examined, except in the southern provinces, where late leafspot and rust were most severe, especially on spanish cultivars. All groundnut cultivars currently grown by farmers are susceptible to early leafspot. Rust was severe on RMP 12. Groundnut rosette was observed only in a few fields and the incidence was very low. It is important to combine rust and leafspot resistance with resistance to rosette in order to achieve yield stability.

Peanut clump and nematodes were important in only a few fields surveyed. Other diseases observed but not considered to be economically important were collar rot, aflaroot, pod rot, leaf scorch, pepper spot, gray spot, *Phyllosticta* leafspot, *Rhizoctonia* leaf blight, witches' broom, spotted wilt, peanut mottle, and a parasitic weed.

### 7.7.2 Niger

A cooperative survey by representatives of ICRISAT, INRAN, Peanut CRSP, and CIRAD was undertaken in the major groundnut growing areas of Niger during the 1987 crop season to assess the distribution and relative importance of groundnut diseases. Fifty-seven fields were examined.

Both early and late leafspots were commonly observed in all fields visited. Late leafspot predominated and was severe in the areas of Say, Dosso, Bengou, Gaya, and Tara. Rust was observed only in Bengou. Pod rot was serious in all drought-affected areas of the country, reducing market value of the produce. Seed and seedling diseases caused reduction in plant stands and yields. Groundnut rosette was epidemic in the major groundnut producing areas.

Peanut clump was widely distributed but was serious only in areas between Zinder and Miria. Other diseases observed during the survey were leaf scorch, gray spot, anthracnose, *Phyllosticta* leafspot,

Rhizoctonia leaf blight, stem rot, peanut mottle, spotted wilt, and witches' broom. These diseases were not observed to be of economic importance.

## 8 TRAINING OUTPUTS (All Degree)

Surname	Sex	University	Department	Degree	Date Degree Received	CRSP Support
<b><u>U.S. Citizens</u></b>						
Parker	M	TAMU	S&CS	PhD		1/4 time, full yr
Durio	M	TAMU	S&CS	MS		1/2 time
Wildman	F	TAMU	S&CS	MS		1/2 time, 5/6 yr
<b><u>Senegal Citizens</u></b>						
N'Doye	M	TAMU	S&CS	M.S.	8/12/88	1/2 time, full yr
<b><u>Burkina Faso Citizens</u></b>						
Ouedraogo	M	TAMU	S&CS	M.S.		1/2 time, 3/4 yr
Pazisma	F	U of Ouga.				Summertime
Kabore	M	U of Ouga.				Summertime

## 9 TRAVEL

### 9.1 Host Country Personnel

O. N'Doye, Senegalese M.S. student at TAMU to present paper at Tulsa, OK (July 12-16) for APRES Annual Meeting and to participate in the CRSP collaborators meeting.

### 9.2 U.S. Personnel

O.D. Smith and W.J. Grichar to Niger (August 27 - September 2), Burkina Faso (September 2-6), and Senegal (September 6-13) to observe research plots and confer with collaborators in each country and ICRISAT scientists at the Sahelian Center.

O.D. Smith, D.H. Smith, C.E. Simpson, W.J. Grichar, and L. Wildman to participate in the APRES Annual Meeting and Peanut CRSP collaborators meeting at Tulsa (July 12-16).

D.H. Smith to Niger and Burkina Faso (September 9-26), and Montpellier, France to participate in collaborative disease surveys with ICRISAT and country scientists in Niger and Burkina Faso, and to confer with Dr. M. Dollet, IRHO-CIRAD, regarding viruses in West Africa.

## 10 PRESENTATIONS

N'Doye, O., O.D. Smith, and G.B. Parker. 1988. Inheritance of earliness in crosses among five early-maturing spanish peanut varieties. Proc. Amer. Peanut Res. & Educ. Soc.: (Abst. in press)

Parker, G.B., O.D. Smith, and W.J. Grichar. 1988. Colorimetric assessment of pod rot disease severity in peanut. Proc. Amer. Peanut Res. & Educ. Soc.: (Abst. in press)

Schubert, A.M. 1988. Peanut production and research in Texas. Annual Meeting, Texas Chapter, Amer. Soc. Agron, Kerrville, Texas.

## 11 PUBLICATIONS

Davis, R.E., D.H. Smith, and A.J. Jaks. 1988. Evaluation of ICRISAT peanut germplasm for resistance to early and late leafspot, 1986. Biol. and Cult. Tests for Control of Plant Dis. 3:64.

- Davis, R.E., W.J. Grichar, and D.H. Smith. 1988. Evaluation of ICRISAT peanut germplasm for resistance to southern blight and pod rot, 1986. Biol. and Cult. Tests for Control of Plant Dis. 3:70.
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## 12 PLANS FOR 1988

### 12.1 Continuing

1. Maintain research into drought resistance of selected peanut lines. Hydraulic press values and relative water content will be further studied as potential indicators of drought tolerance.
2. Select among existing populations and effect additional crosses among parents with resistance to leafspot, stem rot, and pod rot. Rosette resistant parents are being included in some crosses.
3. Develop early-maturing populations for use in host-country selection programs.
4. Evaluate F<sub>3</sub> plant rows derived from a five parent diallel among early maturing peanut cultivars and germplasm lines for agronomic characteristics and earliness.
5. Ascertain disease response and adaptation in Burkina Faso of twenty-two U.S. lines and new accessions from South America.
6. Develop improved disease-assessment techniques.
7. Evaluate 250+ newly collected germplasm lines for reaction to leafspot and soil-borne disease at Yoakum.
8. Continue the WAPEP including the provision of thirty-two entries for increase and preliminary evaluation in host countries.
9. Evaluate selected germplasm for reaction to Tomato Spotted Wilt Virus.
10. Increase seed of additional germplasm lines introduced from Africa and South America.

11. Place seed from convergent crossing program in cold storage for future evaluation.
12. Continue the introgression program to transfer leafspot resistance from wild *Arachis* to cultivated peanut. Backcrossing will continue from both second and third backcross progenies, which exhibit good leafspot resistance.
13. Conduct yield trials on twenty-five lines from the seventh backcross of the introgression program.

#### 12.2 New Thrusts

1. Evaluate germplasm collections from Burkina Faso for foliar and soilborne disease reaction in Texas.
2. Compare field and laboratory reaction to early and late leafspot of partially leafspot resistant, elite lines derived from the introgression program, which utilized early and late leafspot resistant parentage in a bridge cross. Selected components of resistance and comparisons with Southern Runner will be included.
3. Recent outbreak of Sclerotinia blight caused by *Sclerotinia minor* in areas with high temperatures has caused great concern to the Texas industry. Texas spanish-type lines resistant to Sclerotinia blight will be compared *per se* and in crosses under high disease pressure for disease reaction and inheritance of resistance. Crosses will be made to initiate the transfer of resistance to runner-type populations.
4. Data collection and reporting from Burkina Faso will be modified to reduce potential bias from genotypes with poor emergence and high compensation ability.

**Peanut Varietal Improvement for Thailand and the Philippines**

**North Carolina State University—Thailand and Philippines**

**Johnny C. Wynne, Principal Investigator, NCSU**

**INTRODUCTION**

Many of the factors that limit the yield and profitable production of peanuts in Thailand, the Philippines and North Carolina can be overcome by the development and proper management of improved peanut cultivars. Stable cultivars, buffered against the major production constraints—short seasons of annual rainfall, periods of drought, diseases, insects and low soil fertility—are the most economical approach to provide increased and profitable production of an acceptable peanut product. For each priority breeding objective germplasm with the desired variability must be identified or confirmed to be useful in each environment where the crop is to be grown. This requires a coordinated team approach involving breeders, plant pathologists, entomologists, plant physiologists and soil scientists that must identify the best germplasm available to overcome the constraints to production. Germplasm with desired characteristics must then be hybridized with adapted breeding lines in order to develop breeding populations in which selection can be practiced. Selection of potentially useful breeding lines must then be performed in each breeding population. These advanced breeding lines must then be evaluated for yield, stability and other desirable characteristics in multiple location trials. Finally, the superior advanced lines that will be considered for release to farmers must be evaluated for their performance in the appropriate cropping system for which it was selected.

**MAJOR ACCOMPLISHMENTS**

**Thailand**

Two peanut cultivars, Khon Kaen 60-1 and Khon Kaen 60-2, were officially released by the Thai Department of Agriculture. Khon Kaen 60-1, introduced as Mokat, yields 2% more and has larger fruit and seed than Tainan 9. Khon Kaen 60-2, introduced as TMV 3, yields 12% more and has better pod characteristics than SK 38. A large-seeded selection from NC 7 has outyielded the check cultivar Tainan 9 by 20%. This selection has already been recommended for use by Thai growers and will be considered for official release. Cultivar Lampang has been recommended for the before-rice cropping system instead of Tainan 9. Testing is continuing to develop cultivars superior to Lampang for this cropping system.

**Philippines**

UPL Pn-6, locally known as Biyaya 6, was released to growers. Biyaya 6 outyields the check cultivars UPL Pn-4 and BPI Pn-9, has moderate resistance to *Cercospora* leafspot and rust and possesses better seed shape and color than UPL Pn-4. The new cultivar will be used as a primary component of a PCARRD-funded pilot production project designed to increase yields about 50% without significant increases in grower inputs. JL-24, which consistently outyields all other peanut lines in general yield trials, has been seed-increased for testing at selected research stations.

**North Carolina**

NC Ac 18417, a *Cylindrocladium* black rot (CBR)-resistant selection from the cross of NC 8C and Florigiant, was approved for release as NC 10C. NC 10C is expected to replace NC 8C as a component of a program to control the CBR disease. Two breeding lines with good quality and high yielding ability, NC Ac 18411 [a selection from the cross of (FG x NC 5) x (FG x Valencia)] and NC Ac 18423 [a selection from the cross of (FG x FR) x Early Bunch], were determined to be suitable for release to North Carolina growers.

## EXPECTED IMPACT OF PROJECT

This project should result in the development of new and improved peanut cultivars for use in Thailand, the Philippines and North Carolina. These improved cultivars should result in increases of food and vegetable oil in Thailand and the Philippines. Lower production costs should result for North Carolina growers allowing them to compete more favorably in the world market. These results should be accomplished with little or no additional increase in production inputs while reducing unfavorable environmental and human hazards.

## GOALS AND OBJECTIVES

The goals of this project are to (1) develop peanut cultivars with desirable agronomic traits and disease resistances, (2) provide plant pathological information required for the development and utilization of disease-resistant cultivars, and (3) exploit the advantages of these cultivars in the cropping systems used in North Carolina, Thailand or the Philippines.

## ORGANIZATION

### A. U.S. Lead Institution: North Carolina State University, Raleigh

Principal Investigator: Dr. Johnny C. Wynne, Dept. of Crop Science  
 Co-Principal Investigators: Dr. Marvin K. Beute, Dept. of Plant Pathology  
 Dr. H. Thomas Stalker, Dept. of Crop Science  
 Cooperators: Dr. W. V. Campbell, Dept. of Entomology  
 Dr. G. H. Elkan, Dept. of Microbiology  
 Research Associate: Dr. Barbara Shew, Dept. of Crop Science  
 Technicians: Mr. Philip Rice, Dept. of Crop Science  
 Mr. Michael Fitzner, Dept. of Crop Science  
 Ms. Gerry Phillips, Dept. of Crop Science  
 Ms. Joyce Hollowell, Dept. of Plant Pathology  
 Ms. Tracy Jones, Dept. of Crop Science  
 Institutional Representative: Dr. Billy E. Caldwell, Head, Dept. of Crop Science

### B. S.E. Asian Counterpart Institutions: Dept. of Agriculture (DOA), Kasetsart University (KU), and Khon Kaen University (KKU), Thailand; Institute of Plant Breeding (IPB); and University of the Philippines at Los Banos (UPLB), Philippines

Project Coordinators: Dr. Ricardo Lantican, UPLB, Philippines  
 Dr. Crisanto Escano, PCARRD, Philippines  
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 Prof. Andres Pascua, Isabela State Univ., Philippines  
 Mr. Jimmy Domingo, Cagayan State Univ., Philippines

C. USAID Project Officers: Mr. Robert Resseguie, USAID/Manila  
 Mr. Douglas Clark, USAID, Bangkok

## APPROACH

Peanut germplasm is being introduced into Thailand and the Philippines. Observations on agronomic potential, disease and insect resistance, maturity, drought tolerance and other agronomic traits on the introduced germplasm is made in unreplicated nurseries. Selected lines are grown in preliminary replicated tests to identify lines for further testing at multiple locations within each country. In addition to identifying lines for potential release as new cultivars, the tests identify parents for hybridization programs.

Crosses between germplasm with desired traits and locally adapted cultivars are being made to transfer desirable traits to adapted germplasm. Pedigree, backcross and bulk breeding procedures are being used to develop improved cultivars.

Hybrid populations appropriate to the environments of Thailand and the Philippines are being developed at NCSU and in both countries. Late generation material is being evaluated in both countries for potential use. Promising breeding lines will be tested at multiple locations in coordinated yield trials by the DOA in Thailand and by institutions cooperating with IPB in the Philippines.

In addition to the cultivated germplasm, interspecific hybridizations will be utilized to introgress desirable characters from the wild species into *A. hypogaea*. As 40-chromosome populations are developed, they will be incorporated into the *A. hypogaea* breeding programs. Improved cultivars from the breeding projects will be submitted by IPB to the Asian Cropping Systems Network for testing in 11 southeastern Asian countries.

Short visits to both Thailand and the Philippines will be made as needed by the principal investigators to review progress, redefine objectives, plan for the next year and provide technical assistance. Short-term visits of Thai and Filipino collaborators to NCSU, ICRISAT or collaborating countries will be made as needed. Both degree and short-term training will be provided based on need and available funding.

## ACCOMPLISHMENTS IN DETAIL

### Thailand

#### A. Breeding

1. **Department of Agriculture (DOA).** The breeding program at DOA emphasizes selection for high yield, for earliness, for resistance to rust, leafspots, *Aspergillus* spp., A. crown rot and Sclerotinia stem rot, for large-seeded types and for boiling types. Research in 1987-88 included making additional crosses, generation advance, selection in various segregating populations and conducting yield trials of germplasm in various stages of development.

a. **Breeding for high yield.** Ten crosses using Tainan 9, NC 7, Khon Kaen 60-1, Khon Kaen 60-2, PI 119238, (MGS 9 x Chico)-12-16-1 and (MGS 9 x Chico)-12-16-5 were made for high yields. Progenies have been advanced to F<sub>2</sub> generation. Individual plant selection will be practiced among the progenies in 1988.

Selections among F<sub>2</sub> and F<sub>4</sub> generation progenies from six crosses involving Tainan 9, SK 38, Lampang, UPL Pn-4 and Robut 33-1 were made in the dry and rainy seasons of 1987. These selections will be increased for further selection and yield testing.

Yield testing of lines developed for high yield consisted of two preliminary yield trials (37 and 23 test entries), a standard yield trial (13 test entries) and a regional trial (11 test entries). The 37 entries in the preliminary trial were tested at Kalasin in the dry season and at Roi-et in the rainy season. The second preliminary test was conducted at Tha Phra in the rainy season. Eleven entries, all selections from local crosses (Tainan 9 x RCM 387 and reciprocal, Tainan 9 x Ah 24439 and Mokat x Ah 24439), showed superior performance to Tainan 9 in the first trial and four entries from ICRISAT gave higher yield than Tainan 9 in the second test. The standard yield trial, consisting of 13 test entries and 3 checks, was tested at Tha Phra and Kalasin in the dry season. Six entries were selected for the Coordinated Standard Yield Trial in the rainy season. The regional yield trial, consisting of 11 test entries and 3 checks, was conducted at Kalasin, Pitsanulok and Sakon Nakhon in the dry season. Three entries were selected for farm trials and two were entered in the Coordinated Regional Yield Trial in the rainy season.

Medium-seeded lines are tested each year in coordinated yield trials in preliminary, standard and regional tests. The preliminary test, conducted in the rainy season at Roi-et, Mahasarakham and Khon Kaen University, consisted of 27 test entries and 3 checks. Promising entries were (Manipintar x Dht 200)-3B1-1, (Gadjah x PI 314817)-18-1-18, (Mokat x Ah 24439)-12-8-16, (M 145 x NC Ac 17090)-B2, ICG(FDRS)-11, and ICGS(E)-12. (Manipintar x Dht 200)-3B1-1 also had low scores for rust and leafspots. Two sets of coordinated standard yield trials were conducted. The first set, the 1986 yield trial which consisted of 13 test entries and 3 checks, was conducted in the dry season at Kalasin and Pitsanulok. Two entries [(MGS 9 x Robut 33-1)-5-2 and ICGS-4] were advanced to the 1987 coordinated regional yield trial and three [(FG x NC Ac 17090), No. 77, and (Panjab x PI 314817)-15-2-34] were retained in the 1987 coordinated standard yield trial. The 1987 coordinated standard yield trial consisting of 11 test entries and 3 checks was conducted in the rainy season at Nakhon Sawan, Pitsanulok, Roi-et, and KKU. Promising entries were (Panjab x PI 337394F)-4, No. 77, (Taiwan x PI 337394F)-2, PI 268659, (M 13 x Dht 200)-3-B1-3 and (M 13 x Dht 200)-3-B1-2. The 1986 and 1987 regional yield trials were conducted in the dry and rainy seasons, respectively. The 1986 trial consisting of 11 test entries and 3 checks was conducted at Chinat, Kalasin and Sakon Nakhon. Six entries were selected for the 1987 trial which was conducted in the rainy season at Chiangmai, Rayong, Srisamrong and KKU. The best yielding entry was (Gadjah x PI 314817)-8-1-18 which averaged 41% more than Tainan 9.

Four lines (Mokat, TMV 3, RCM 387 and Tainan 9) were tested in farmers' fields at Pitsanulok, Ubol, Khon Kaen, Mahasarakham and Chiangmai in the dry season and at Kalasin, Pitsanulok, Loei and Nakhon Sawan in the rainy season. Mokat gave 1.6 and 11.8% higher yield than Tainan 9 in the dry and rainy seasons, respectively, while TMV 3 outyielded Tainan 9 by 11.4 and 5.7%.

b. **Breeding for earliness.** Selection for early maturity has the objective of developing high yielding cultivars which mature in 80-85 days for use in specific cropping systems. Segregating populations undergoing generation advance and selection were progenies from crosses of Tainan 9, SK 38, Lampang, UPL-Pn4, NC 2 and Ga 119-20 with Chico. Yield trials of early maturing lines included three preliminary, a standard, a regional and a farm trial. A preliminary yield trial of ICGS(E) lines was conducted at Tha Pra and Roi-et in the rainy season. Promising entries will be selected for further evaluation after 2 years of testing. The standard yield trial was conducted at three locations but poor yields were obtained at all locations due to drought stress at the late growth stage. The regional yield trial was conducted in the late rainy season at Roi-et, Kalasin and Pitsanulok. (MS9 x Chico)-12-13-3 and M 10 were selected for further testing in the rainy season regional trial along with three other lines selected from other trials and three checks. The rainy season trial was conducted at Banmai Samrong, Roi-et, Mahasarakham and Loei. All test entries outyielded the checks.

Two early lines were tested in the farm trial with checks Tainan 9, SK 38 and Lampang at Khon Kaen, Pitsanulok, Ubon, Kalasin, Mahasarakham and Surin in the dry season. Only one line, (MGS 9 x Chico)-12-16-1, was tested in the rainy season at Khon Kaen, Rayong, Loei, Roi-et, Petchaboonand and Sakon Nakhon. It gave equivalent pod yields to the checks but had a higher shelling percentage, thus higher seed yield.

c. **Breeding for rust and leafspot resistance.** In 1987, 355 F<sub>2</sub> plants from 12 crosses involving NC Ac 17027, NC Ac 17090, and PI 298115 were selected during the dry season at Tha

Phra. Three hundred seventy-nine  $F_3$  plants were selected during the rainy season. Pedigree selection will continue in these populations.

d. **Breeding for resistance to *Aspergillus* sp.** There are three groups of materials undergoing selection and yield testing for resistance to *Aspergillus* sp. The first group contains progenies from 51 crosses involving 18 resistant lines with seed coat resistance (J11, Faizpur, Var. 27, Ah 7223, PI 337394F, PI 337409, Monir 240-30, UF 71513-1, U-4-47-7, C55 x 437, NC Ac 841, AR-1, AR-2, AR-3, AR-4, Sunbelt Runner, GFA-1, GFA-2) crossed with Tainan 9, NC 7, NC 8C and SK 38. Two hundred fifty-five  $F_3$  lines were selected and grown in the rainy season. Of these, 33 were selected for subsequent yield evaluation. Twenty lines were tested in a preliminary yield test at Tha Phra in both the dry and rainy seasons. Four lines (J11 x RCM 387)-7-1-10, (J11 x Ah 24439)-18-11-16, (J11 x Ah 24439)-26-13-2 and (J11 x Mocket)-2-5-8 were selected for further yield and resistance evaluation. Three lines selected for *Aspergillus* resistance were compared in farmers' fields with Tainan 9 at Khon Kaen, Kalasin, Pitsanulok and Changmai in the dry season and at Khon Kaen, Ubon, Rayong, Loei, Petchaboon and Roi-et in the rainy season. Two lines, (Mocket x J11)-12-2-25 and Lampang x J11-12-2-29, performed well agronomically. Resistance to *Aspergillus* for these two lines is being evaluated.

e. **Breeding for resistance to *Aspergillus* crown rot.** Screening for resistance to *Aspergillus* crown rot began in 1985 by screening 155 lines received from NCSU in a heavily infested field at Tha Phra. Forty-nine lines with low disease incidence were yield tested in the dry and rainy seasons of 1986 and evaluated for *Aspergillus* crown rot resistance in the greenhouse. Thirteen lines were selected and further evaluated in a standard yield trial in both the rainy and dry seasons of 1987. High yielding entries with some degree of *Aspergillus* crown rot resistance were  $F_8$  (G 37 x PI 259747), (Ah 65 x NC Ac 17090), PI 269008 and PI 270835. These lines, however, had smaller seed size than Tainan 9. Another promising entry was 82  $F_8$  L126 which had the largest seed size in this trial. This line outyielded Tainan 9 and had the same level of disease incidence as Tainan 9 which also showed some resistance to *Aspergillus* crown rot.

f. **Breeding for resistance to *Sclerotinia* stem rot.** In 1985, 12 crosses were made by crossing two resistant lines (NC 2 and Ga 119-20) with high yielding lines. The  $F_2$ s were screened in a field with high incidence of stem rot at Tha Phra in 1986. The  $F_3$ s were grown in the dry season of 1987, and 377 plants were selected. The  $F_4$ s were grown plant to row in a field inoculated with *Sclerotium rolfii* in the 1987 rainy season. Two hundred fifty plants were selected for subsequent evaluation.

Screening for resistance to *Sclerotinia* stem rot has also been done on 1148 lines received from NCSU. Through successive screenings, 29 resistant lines were selected and evaluated in the diseased fields in the rainy season of 1987. The lines showing stem rot resistance and good yield level were PI 268742, PI 261921, UF 78305, Southern Cross, PI 262016 and PI 268972.

g. **Selection of boiling-type peanuts.** The  $F_1$ s and  $F_2$ s from eight crosses between a line with long and large 3-4-seeded pods and sweet taste [NC Ac 17135 (AF)] and large-seeded and high yielding lines (Mocket, 68 SAN 63, NC Ac 17093, NC Ac 17127, NC Ac 17090, Shulamit, NC Ac 16020, and a local cultivar from Chongsarika) were grown in the dry and rainy seasons, respectively. A total of 377  $F_2$  plants were selected for further advance and selection. Four yield trials of boiling-type lines were conducted in 1987. In the dry season a preliminary yield trial of 27 lines and 3 checks was conducted at Tha Phra and a standard yield trial of 43 entries was tested at Chiangmai, Pitsanulok and Kalasin. Six lines from the preliminary yield trial were advanced to the standard yield trial stage in the rainy season and seven lines from the standard yield trial were entered in the rainy season regional yield trial. The rainy season standard yield trial of 12 entries was grown at Banmai Samrong, Mahasarakham and Mukdaharn. Only the line (Tainan 9 x Ah 24439)-7-9-13 gave high fresh pod yield. The rainy season regional yield trial (10 entries) was conducted at Khon Kaen, Ubon, Roi-et and Loei. Three lines gave high fresh pod yields and good pod characteristics. These lines were Local from Nong-Bua-Dang, (Taiwan 2 x UF 71513-1) and (Mocket x UF 71513-1).

h. **Improvement of large-seeded types.** Two yield trials of large-seeded lines were conducted in 1987. The first trial consisting of 10 test entries and a check was the Standard Coordinated Yield Trial and the second trial consisting of 13 test entries and a check was the

standard yield trial of large-seeded lines selected from materials received from NCSU. The Coordinated Standard Yield Trial of large-seeded lines was conducted in the dry season at Kalasin and Pitsanulok. None of the test entries gave higher yield than Tainan 9. Among the test entries (Shulamit x PI 337394F)-1 gave the highest yield (1475 kg/ha compared to 1612 kg/ha of Tainan 9) and had the largest seed size (56.7 g/100 seeds). The standard yield trial of lines from NCSU was grown at Khon Kaen and Chiangmai in the dry season and at Khon Kaen, Chiangmai and Nakhon Sawan in the rainy season. The line NC 7 (selected version) gave the largest seed size and showed the best yield performance among the large-seeded entries. Due to a great demand for large-seeded peanut lines, the line NC 7 (selected version) has now been entered in the farmers' field trials. Although the line has not been officially released, it is recommended as a large-seeded Virginia type.

NC 7 (selected version) was derived from selection within the cultivar NC 7. In 1983, 1416 lines were received from NCSU, one of which was NC 7. These lines were grown for initial adaptation to Thailand at the Kalasin Field Crops Experiment Station in the rainy season of 1983 and in the dry season of 1984. The lines were again grown in the 1984 rainy season at the Khon Kaen Field Crops Research Center at Tha Phra. NC 7 was among the lines selected for further evaluation. Selection within NC 7 gave 20 plants which showed good pod filling and were free from Sclerotium blight. Bulk seeds from these selected plants were grown for seed multiplication and further selection in the dry season of 1985. Plants with good pod filling, less spreading, high number of pods and free from Sclerotium blight were selected and the seeds were bulked for use in yield evaluation. The selected version of NC 7 was then tested in the preliminary yield trial in the 1985 rainy season, 1986 dry and rainy seasons and in the standard yield trials in the 1987 dry season (two locations), 1987 rainy season (three locations) and 1988 dry season (two locations). It was also tested in the farm trials in the 1987 rainy season (9 plots, 6 provinces) and in the 1988 dry season (15 plots, 10 provinces). NC 7 Selected has yielded about 20% more than Tainan 9.

i. **Release of new cultivars.** In 1987 two new cultivars, Mocket and TMV 3, were officially released by DOA. Mocket was released as "Khon Kaen 60-1" to replace Tainan 9 and TMV 3 was released as "Khon Kaen 60-2" for boiling.

Mocket was originally received by the DOA in 1974 as an entry in a trial sent from SEARCA for testing in Thailand. This cultivar showed good performance in the 1974 trial and was selected for subsequent testing. From 1975-80, Mocket had been tested in various yield trials of the DOA totaling 31 tests, 9 seasons and 8 locations. On the average, Mocket gave 2% higher yield than Tainan 9 in the rainy season. However, it has larger seed size than Tainan 9. During 1982-86, it was tested in farm trials totaling 35 tests covering 11 provinces and 9 seasons. Over 20 tests in the rainy season and 15 tests in the dry season, Mocket outyielded Tainan 9 by 6% (rainy season) and 3% (dry season). During 1975-84, Mocket was tested in 19 tests by KKU and gave 14% higher yield than Tainan 9. In general, Mocket has similar characteristics to Tainan 9 except that it has larger seed size.

Khon Kaen 60-2 was derived from work of the DOA on selection for boiling types. The work began in 1979-80 by selecting lines with 3-4 seeds/pod and high yield. From 465 lines in the collection, 84 were selected and subsequently grown in plant to row for further selection. Thirty-seven lines were selected for testing in 1981. TMV 3 (designated as KAC 118) was identified as a promising line which gave substantially higher yield than the check entry SK 38. It was then advanced to the standard yield trial (1981-83), the regional yield trial (1983-85), and farm trial (1985-86), respectively. From these trials TMV 3 gave an average fresh pod yield over 32 tests 12% higher and an average dry pod yield over 21 tests 8% higher than those of the boiling-type check SK 38. It also has better pod characteristics for boiling than SK 38.

2. **Khon Kaen University (KKU).** Breeding work at KKU continued for resistance to rust and leafspots, for earliness, for high yield, and for growing before and after rice without irrigation. The work included generation advance and screening of several groups of segregating materials received from ICRISAT and NCSU and yield testing of lines selected for the desirable traits respective to the individual breeding objectives. New crosses were also made locally and some were received from NCSU. Studies were conducted on the inheritance of leafspots and their components and on growth and development of lines with different maturities in the different growing seasons. Collaboration was continued with ICRISAT in testing drought-tolerant lines under the after-rice unirrigated condition and with DOA in conducting coordinated yield trials.

a. **New crosses made and new materials received.** During 1987, 83 crosses were made among 25 parental lines with different desirable traits to generate segregating materials for further selection. Two germplasm lots were received from NCSU in 1987. The first lot contained 20  $F_2$  lines resistant to late leafspot and the second lot was comprised of  $F_2$  progenies of six crosses involving Tainan 9.

b. **Breeding for the after-rice unirrigated condition.** Fourteen yield trials of lines were conducted at KKU under the after-rice unirrigated condition in the dry season of 1986-87. These included 2 advanced yield trials, 4 intermediate yield trials, 7 preliminary yield trials, and a test of drought-tolerant lines from ICRISAT. The test entries excluding checks totaled 346 lines. In addition, 58 progenies from crosses to a drought-tolerant line were grown for selection, and a farmer field trial of eight lines was conducted at Ban Kog Yai, Amphur Banfang and Khon Kaen.

Yields obtained were high in all trials, with the highest yielding entry yielding more than 2 tons/ha in most of the trials. In all but two trials there were several entries showing superior performances to Tainan 9. However, some of these high yielding lines have poor pod characteristics, particularly drought-tolerant lines from ICRISAT. Only good performing lines with acceptable pod characters were selected for subsequent testing. Line No. 8 from the farmer trial gave the highest pod yield but had a high percentage of shrivelled seeds. High yielding entries without shrivelled seed problems were ICGS 44 and (X 52-x-x-3XB x Chico)-8-1-1.

c. **Breeding for the before-rice condition.** Six yield trials of lines were conducted at KKU in the 1987 before-rice growing season. These included an advanced yield trial, 2 intermediate yield trials and 3 preliminary yield trials. The test entries excluding checks totaled 148. In all trials there were several entries showing superior performances to Tainan 9 and some were earlier in maturity than Tainan 9. High yielding entries which have been tested for 2-3 years will be selected for multi-locational testing. The cultivar Lampung which has been included as a check in many trials has shown good and consistent performances over the past few years. This cultivar outyielded Tainan 9 in most of the trials under the before-rice conditions. It is suggested that this cultivar be recommended as a stop-gap cultivar for the before-rice cropping system.

d. **Breeding for earliness.** Work on breeding for early maturity at KKU in 1987 consisted of an advanced yield trial, an intermediate yield trial and a preliminary yield trial. Most of the entries tested were early lines from ICRISAT. The advanced yield trial was conducted at three locations (KKU, Roi-et Field Crops Experiment Station and Mahasarakham Field Crops Experiment Station). The intermediate and preliminary yield trials were conducted only at KKU. All trials were conducted in the rainy season.

Moderate yields were obtained from the trial at Roi-et, but low yields were obtained at KKU due to drought stress at pegging stage. Yields from the trial at Mahasarakham were very low and quite variable. In the advanced yield trial at Roi-et, five entries gave higher yields and were 7-10 days earlier in maturity than Tainan 9. The top yielding entry was ICGS(E)-31. Several entries in the advanced yield trial at KKU gave superior performances and were earlier in maturity than Tainan 9. The top yielding entries were ICGS(E)-12 and (MGS x Robut 33-1)—5-2-2. These two entries had the same maturity as Chico and were 20 days earlier than Tainan 9. Several entries in the intermediate yield trial also showed superior performances and were earlier in maturity than Tainan 9 but superior entries in the preliminary yield trial had the same maturity as Tainan 9.

e. **Breeding for resistance to rust and leafspots.** Work on breeding for resistance to rust and leafspots in 1987 included generation advance of  $F_2$  progenies from foliar disease-resistant crosses, screening of  $F_4$  progenies in disease nurseries, and three yield trials of foliar disease-resistant lines. The advanced yield trial was conducted at KKU and the Khon Kaen Field Crops Research Center at Tha Phra; the intermediate yield trial and the trial of foliar disease-resistant sources were conducted at KKU.

Yields obtained from the advanced yield trial at KKU were rather low but were quite high at Tha Phra as the trial at Tha Phra was irrigated. Several entries gave higher yields than Tainan 9 at both locations and some showed a moderate degree of leafspot resistance. Most entries in the intermediate

yield trial gave higher yield than Tainan 9 (more than 100%), although the yield level was rather low. Poor yields were obtained from the trial of disease-resistant sources.

f. **Breeding for high yield.** Ninety-two lines in the high yielding groups were tested in six trials in the rainy season of 1987. The trials included an advanced yield trial, two intermediate yield trials, a repeated testing of the 1985 International Peanut Evaluation Program (INPEP) Trial, and two preliminary yield trials. In addition, three coordinated yield trials (regional, standard and preliminary) of medium-seeded lines were also conducted.

The advanced yield trial was conducted at KKU and Khon Kaen Field Crops Research Center at Tha Phra. Yields at KKU were rather low due to drought stress at pegging stage, but yields at Tha Phra were high as the trial was irrigated during the dry spell. Superior entries in this trial were ICGS 52 and ICGS 13-1 which gave yields considerably higher than Tainan 9 at both locations; however, ICGS 52 has small seed.

Several entries in the intermediate and preliminary yield trials showed yield superiority over Tainan 9, although the yield levels in these trials were low. One of the intermediate yield trials was also tested at Mahasarakham, but the yields obtained were very low. Low yields were also obtained for all entries in the INPEP trial.

## B. Pathology

1. **Department of Agriculture (DOA).** Peanut pathological work at the DOA in 1987 concentrated mainly on the evaluation of lines for resistances to rust (*Puccinia arachidis*), stem rot (*Sclerotium rolfsii*) and crown rot (*Aspergillus niger*) under greenhouse conditions. Most of the lines tested were previously identified as resistant to the respective diseases under field condition. Yield loss caused by peanut stripe virus was also assessed by the Plant Virology branch.

a. **Reactions of lines to rust.** Twenty-three lines were evaluated for rust resistance in a greenhouse in Bangkok. Tainan 9 was used as a susceptible check and NC Ac 17090 as a resistant check. In each line, 20 plants grown in four clay pots were inoculated with spore suspension of *Puccinia arachidis* ( $1.1 \times 10^5$  spores/ml) at 30 days after planting. The plants were kept inside a polyethylene chamber for 48 hours after inoculation. At 85 days, disease was rated using a 1-9 score. Out of 23, only eight lines [ICG(CS)S 18, 19, 20, 21, 49, 16 and 68 and M-13] showed a moderate level of resistance having disease scores lower than 6.5. Tainan 9 was severely diseased while infection on NC Ac 17090 was considerably low.

b. **Reaction of lines to stem rot (*Sclerotium rolfsii*).** Reactions of 127 lines to *Sclerotium rolfsii* were evaluated in a greenhouse at DOA. The lines tested were selected from those that gave high yield and showed some degrees of stem rot resistance in the previous year. Screening was done by growing the plants in *Sclerotium rolfsii*-infested soil. The soil was sterilized and put in polyethylene bags prior to *Sclerotium* inoculation. Disease ratings were done at 10-day intervals until the plants were 80 days old. At that age most of the plants were killed except NCSU 1291 which had 33.3% disease incidence. Other lines showing resistance were NCSU 324, NCSU 437, NCSU 761, NCSU 829 and Tainan 9. In these lines, 53.3% of the plants were killed by the disease.

c. **Reactions of lines to *Aspergillus* crown rot.** Fourteen lines which have shown good yield performances and field resistance to crown rot and a susceptible check, Tainan 9, were artificially inoculated with *Aspergillus niger* spore suspension. For each line, three pots each of 10 seeds were inoculated and another three were left uninoculated as a control. Crown rot incidences were recorded at 10 days intervals until the plants reached maturity. The disease occurred in both the inoculated and uninoculated pots, but higher incidences were observed in the inoculated pots in all the lines except PI 269008 and PI 270835. For these two lines the disease incidences in the inoculated and uninoculated pots were the same, indicating some resistance to late infection.

d. **Distribution of virus disease in northeast Thailand and their effects on yield.** Surveys on the prevalence of peanut virus diseases in the northeast were conducted in Kalasin, Khon Kaen, Roi-et, Loei and Udorn Thani during 1987. Results showed that 41.6% of the peanut plants in farmer fields and in research stations were infected by peanut yellow spot virus, 23.2% by peanut stripe

virus (PStV), and 14% by tomato spotted wilt virus. Ten-plant samples of Tainan 9 inoculated with PStV were compared with the noninoculated control in 10 replicates. Inoculation with PStV caused 10.9% reduction in fresh pod weight and 18.7% reduction in dry seed weight. However, these losses were not statistically significant.

**2. Khon Kaen University (KKU).** Since peanut stripe virus has been shown to be of economic importance, in 1987 pathological research at KKU has placed more emphasis on studying this disease. Work in 1987 included the assessment of its effect on yield, its biology and epidemic ecology. In June 1987, a coordinators' meeting was held in Malang, Indonesia, bringing together the scientists from all countries concerned with PStV. Several recommendations were made regarding future direction of research and cooperations among countries and institutes working on PStV. It was also planned that virus characterization would be undertaken at Montpellier in France by Dr. Sopone Wongkaew of KKU.

**a. Yield loss assessment for peanut stripe virus.** Five peanut cultivars (Tainan 9, SK 38, Lampang, Mocket, and RCM 387) were assessed for yield loss due to PStV infection under field conditions at Khon Kaen. The plants were mechanically inoculated with the ring spot variant of PStV propagated in KC-84 R cowpea at 2 weeks after emergence using a high pressure spray-gun. After 3 weeks, 40-50% of the plants in the inoculated plots became infected, but none was detected in the uninoculated plots. Natural virus spread started to occur at 4 weeks and, when the plants reached 80 days, the numbers of infected plants in the inoculated and uninoculated plots were not different. The virus appeared to slightly reduce pod yields of SK 38, Mocket and Lampang but had no effect on Tainan 9 and RCM 387, although it increased the number of shrivelled seeds in Tainan 9 considerably. Among the five cultivars tested, RCM 387 showed the highest level of tolerance to PStV infection as its seed quality and pod yield were not affected.

**b. Effect of insecticide sprays on the distribution of peanut stripe.** The effect of insecticide sprays on the spread of peanut stripe was investigated by applying monocrotophos at 14-day intervals to 22 lines used in previous experiments. The first spray was done at 30 days after planting. The stripe disease incidences in both the sprayed and nonsprayed (control) plots assessed at 75 days were too low to make meaningful comparisons.

**c. Purification and serology of peanut stripe.** This experiment was conducted to compare the efficacy of procedures currently employed for potyvirus purification and to investigate the serological relationship among PStV symptom variants and other legume potyviruses. Antiserum prepared from purified PStV-R (ringspot variant) reacted strongly with the purified preparation and with extracts taken from PStV-R and PStV-M (mild mottle variant) infected leaves treated with 1.5% SDS. The test was done in an agar gel double diffusion system. Extracts from blackeye cowpea mosaic virus (BICMV) and the PStV-G (green-blotch variant) infected leaves reacted only weakly to the PStV-R antiserum. Extracts from both soybean mosaic virus (SMV) infected leaves and healthy plants gave a negative result. By using a reciprocal test in which antisera of peanut chlorotic ring mottle (PCRMV), SMV, BICMV and peanut mottle virus (PMV) were used as references, all PStV variants appeared to react strongly with the antisera of PCRMV and BICMV. A mild reaction with spur formation was observed between the wells that contained purified preparation of PStV-R and SMV-antiserum, indicating a distant relationship between the two viruses. No relationship was observed among these viruses and PMV.

**d. Evaluation of a modified technique for establishing a field disease nursery for screening lines resistant to foliar diseases.** A technique for establishing a field disease nursery was modified and evaluated. Instead of preplanting a susceptible cultivar one month prior to the test entries, early susceptible lines previously identified were used as infector rows and were planted at the same time as the test materials. Combined scores of the three foliar diseases obtained at 80 days on selected cultivars were found to be comparable to those recorded in the earlier screenings, indicating that the modified technique was equally effective as the previous technique. The modified technique will be used in future screening because it is simple and more economical.

**e. Peanut diseases survey in the dry season.** A trip was made to growing areas in four provinces in the northeast during late February 1988. Altogether, 10 locations in Kalasin, Roi-et, Surin and Buriram were inspected. The survey covered both irrigated areas and nonirrigated areas

where peanut was grown on residual soil moisture. Seedling blight (*Aspergillus niger*) combined with stem rot (*Sclerotium rolfsii*) appeared to be the most important disease found in the irrigated areas followed by bud blight (tomato spotted wilt virus). The incidences of 3-10% were normally observed for these diseases in most fields. In contrast, the disease incidences in the nonirrigated areas were so low that they were considered insignificant. Regardless of the locations, thrip injuries appeared to be the most prominent abnormality detected on most plants.

3. **Kasetsart University (KU).** Emphasis of peanut pathological research at KU is on late leafspot disease. The potential of biological control of this disease was investigated using microfloras found in proximity to peanut plants. Studies were also conducted on natural occurrence and accumulation of aflatoxin in peanut and on the role of inoculum on toxin production.

a. **Feasibility of using phylloplane and rhizosphere microfloras as a biological control agent.** The feasibility of using microflora from the aerial part of the plant has been demonstrated in peanut. These microflora could be isolated from leaves or soils. The role of phylloplane and rhizosphere microflora as a biological control agent (bca) against black leafspot and other diseases of peanut was investigated at KU in 1987. Leaf washing, leaf imprinting and spore fall methods were used to isolate phylloplane microflora. Samples of leaves and soils were collected from three peanut fields at Bangkhen campus, Kamphangsaeen campus and at Suwan Farm. Isolations were done during the rainy season of 1987 (June-November). The microflora were identified, characterized, categorized and cultivated on PDA for further use. Their propagules were multiplied and tested for their efficacy as a bca against the late leafspot causal organism by post-inoculation on detached leaves in the laboratory as an initial screening for those to be used in field trials.

Results revealed that the predominant and commonly found microflora on leaves and in soils were *Aspergilli*, *Penicillia*, and *Fusaria*. Soil plating technique yielded a different group of organisms from the phylloplane microflora. The organisms isolated by soil plating technique comprised mostly bacteria and lower fungi (e.g., *Rhizopus* sp. and *Mucor* sp.), whereas the phylloplane microflora were Deuteromyceteous fungi (e.g., *Alternaria* spp., *Cladosporium* sp., *Drechslera* sp., *Curvularia* sp. and the three predominant genera mentioned above). *Trichoderma* spp. and *Chaetomium* sp. were additional species found in the rhizosphere. The best method for phylloplane isolation was leaf imprinting.

Results of the preliminary test on the efficacy of these microflora as bca in the laboratory indicated that some genera of *Fusaria* and *Penicillia* effectively reduced the lesion number of black leafspot to less than five lesions per leaf compared to more than 20 lesions per leaf obtained from the check. However, the number 9 isolate of *Fusarium* sp. appeared to be ineffective in reducing the development of black leafspot. A field trial of some of these microflora as bca is currently underway.

b. ***Aspergillus flavus* in peanut.** Work on aflatoxin in peanut at KUU in 1987 included studies on natural occurrence of the toxin, accumulation of the toxin in various kernel parts, and the role of soil inoculum of *Aspergillus* sp. on aflatoxin production. To assess the aflatoxin contamination in peanut grown in various parts of the country, seeds were collected from different locations, mostly from the north and northeast, both in the dry and rainy seasons. The samples were analyzed for aflatoxin contamination using a Velasco Aflatoxin meter and were observed for colonization of *Aspergillus* sp. The results indicated a positive correlation of colonization of the fungus (%) and aflatoxin content (ppb) regardless of seed moisture content and the location where the sample was collected.

Kernels and roasted peanuts (conventional roasting technique used in the villages before grinding to make ground peanut for local consumption) were collected from Kalasin province in the northeast. Roasted pods were split, then seed coat, cotyledon, and embryo were collected separately. Aflatoxin levels in different parts were determined using a Velasco Aflatoxin meter. Data obtained indicated that, although moisture contents were generally low (10.36-14.56%), colonization of *A. flavus* on different parts of the kernels varied greatly ranging from 7.0-46.0%. From aflatoxin content determination it was apparent that most of the toxin was accumulated in the embryo (274 ppb) and in ground cotyledon (275 ppb).



A study was conducted to determine the role of *Aspergillus* sp. as soil inoculum on aflatoxin accumulation in peanut. The peanut cultivar Tainan 9 was planted in three-row plots. When the plants were 2 and 3 months old, the plants in the middle row of each plot were inoculated with *Aspergillus* sp. at different rates. The inoculum was prepared from the fungus cultured in 9:1 of soil and corn meal medium, autoclaved and incubated for 2 weeks. At harvest, soils from the middle rows were collected to determine the amount of *Aspergillus* propagules in the soils. Pods were collected from 10 plants in each middle row and then were threshed and stored for later determination of aflatoxin content by the Velasco Aflatoxin meter. Results revealed that there was a significant positive relationship of propagule numbers in the soil with toxin accumulation in kernels and with kernel infection percentage. In general, the higher the inoculum dosage used (2.0-2.5 kg/row), the more numbers of propagules found in the soils ( $30 \times 10^4$  -  $50 \times 10^4$  propagules/g soil) and the higher level of toxin detected in the kernels (20-24 ppb).

### Philippines

**A. Germplasm Collection and Screening.** One hundred thirty new accessions were collected from NCSU, ICRISAT, Indonesia and the Philippines. These newly introduced materials are sources of desired genetic traits such as early maturity, resistance to late leafspot, stem rot, leafhopper, spider mites and tolerant to drought conditions. Some  $F_2$  seeds of six NCSU crosses (Chico x Tainan 9, NC 7 x Tainan 9, 73-30 x Tainan 9, 28-206 x Tainan 9, 55-437 x Tainan 9 and 703-80 x Tainan 9) were obtained in 1987. The female parents were selected for earliness and large seededness. These materials are currently grown in the field for initial observation of desired traits.

Two hundred sixty accessions were screened during the 1987 wet season. Seed yield ranged from 0 to 2.12 tons/ha. The top yielders were NC 17133 RF (2.12 tons/ha), PI 259693 (1.88 tons/ha) and PI 259693 (1.70 tons/ha). Fifty-three new accessions from NCSU were tested for resistance to stem rot under greenhouse conditions. Initial results showed that 28 genotypes were not infected, 23 had moderate resistance, and 2 were susceptible to stem rot (*Sclerotium* wilt).

**B. Hybridization and Generation Advance.** Forty-two crosses and two backcrosses were completed in January 1987. An additional 36 crosses involving five high yielding female parents and 18 male parents (resistant/ tolerant to rust, stem rot, late *Cercospora* leafspot, leafhopper, acidic soils) were completed from February to June 1987. A high yielding cultivar from Thailand and a local cultivar (UPL Pn-4) were also crossed. Fifty crosses involving eight backcrosses and 42 two-way crosses are still in progress.

Seventy populations in various segregating generations (12  $F_4$ , 29  $F_3$  and 29  $F_2$ ) were advanced during the 1986-87 dry season. During the 1987 wet season, 979 individual plants were selected from 12  $F_3$  families. These plant selections are now grown in observational nurseries for the 1987-88 dry season. Populations in various segregating generations (29  $F_5$ , 14  $F_4$ , 27  $F_3$ , 36  $F_2$ ) are currently planted under upland conditions (1987-88 dry season).

**C. Yield Testing and Seed Production.** Three sets of yield trials were conducted at UPLB during the 1987 dry season (January-May). The first set consisted of 49 entries and was conducted at IPB and Isabela State University. Only three entries (IPB Pn 82-70-53, IPB Pn 82-70-59 and IPB Pn 82-70-64) performed consistently well at both locations. The second set of yield trials consisted of 100 entries. Only three IPB elite lines (IPB Pn 82-68-97, IPB Pn 82-70-29 and IPB Pn 82-70-38) were resistant to stem rot among the 10 top yielding entries. IPB Pn 82-80-17 was the highest yielder (1.14 tons/ha) but was only moderately resistant to stem rot. The third set of preliminary yield trials had 144 entries. The high yielding entries were IPB Pn 83-116-68 and IPB Pn 82-71-44. Four peanut lines (IPB Pn 82-71-54, IPB Pn 82-75-6, IPB Pn 82-68-145 and IPB Pn 82-116-53) were rated as immune to stem rot under natural field infection.

Twenty entries were tested in the general yield trial (GYT) conducted at four locations (IPB, ISU, USM and CSU) during the 1986-87 dry season. The trial at ISU failed because of poor stand. JL-24 demonstrated a consistently superior pod and seed yield among all other peanut lines in the GYT at the three stations. The other promising high yielding entries were Robut 33-1, ICG(FDRS) 10, ICG(FDRS) 11, NC Ac 17070, RLRS 2 and RLRS 12. ICG(FDRS) 10 and ICG(FDRS) 19 were resistant to stem rot. These promising materials are being seed increased for inclusion in advanced

yield trials. In the advanced yield trial conducted during the 1987 dry season, low yields were obtained because of severe damage by *S. rolfisii*. Only IPB Pn 1-174 was resistant to stem rot.

**D. Breeding for Disease Resistance.** Twenty-one selected peanut cultivars/lines were inoculated for rust resistance by a scalpel-leaf-dip method in the greenhouse. Five advanced lines (IPB Pn 82-71-27, IPB Pn 82-68-16, IPB Pn 82-68-174, IPB Pn 82-70-67 and IPB Pn 82-70-27) had no infection, while six entries (IPB Pn 82-51-21, UPL Pn-6, B-150, IPB Pn 82-75-45 and IPB Pn 82-45-28) were moderately resistant.

Six accessions (FESR 2, FESR 4, CES 3-2, UPL Pn-4, CES 101 and BPI P9) having different disease reactions to rust were used to study the morphological basis of resistance. Results showed that the number of stomates per unit leaf area was significantly higher in susceptible entries (CES 101 and BPI P9) than in resistant entries (FESR 2 and FESR 4). However, the six entries ranked inconsistently based on the size of stomatal aperture. Based on the number of trichomes per microscopic field, resistant entries generally had more trichomes than susceptible entries. Peanut cultivars with significantly higher cutin were not necessarily more resistant when compared with other cultivars.

Six peanut lines in the observational nursery were resistant to late leafspot. These were IPB Pn 83-113-21, IPB Pn 83-114-115, IPB Pn 83-114-116, IPB Pn 83-116-2, IPB Pn 83-116-3 and HYQ (CG)S-5. In the germplasm screening of 260 promising accessions, two accessions (69-101 Senegal and RMP-12) were resistant to late leafspot.

One hundred thirty promising peanut cultivars were tested for resistance to stem rot infection under greenhouse conditions. Results showed that 84 were highly susceptible and 11 were susceptible; 35 had poor germination and were not scored.

Plants infected with bacterial wilt were collected from Isabela and UPLB. *Pseudomonas solanacearum* was isolated and pathogenicity was tested. For race identification of the isolates, *P. solanacearum* was inoculated on tomato, eggplant, tobacco, pepper and abaca. Response of host plants to inoculation of *P. solanacearum* showed that the isolates from Isabela and UPLB belong to race 1. Biochemical tests on peanut isolates from Isabela showed that the isolate belongs to Biovar 3.

**E. Breeding for Insect Resistance.** Three hundred fifty entries were evaluated for resistance to leafhopper, leaf defoliators and pod borers under field nursery conditions. Of the eight entries in the advanced yield trial, only E.G. Pn 48 was consistently resistant against the major insect pests under study. Individual plant selections from the NCSU F<sub>3</sub> materials were made on the basis of desirable agronomic characters and resistance to leafhoppers and defoliators. The selected materials will be further evaluated for pest resistance and yield potential.

In an effort to assist in developing strategies to prevent and/or minimize the spread of PSTV, one ICRISAT cultivar (ICGS 5420) which was resistant to *Aphis craccivora* (the aphid reported to be an efficient vector of PSTV) was evaluated. Vector resistance and percentage peanut stripe virus infections were monitored. Preliminary data indicated that ICGS 5420 is also resistant to the local *Aphis craccivora*. Compared to UPL Pn-4, ICG 5420 had no PSTV infection, while the local cultivar had as much as 35.7% infection. While ICG 5420 showed a good level of resistance to the aphid, it should be carefully evaluated because in the international insect resistance nursery it appeared to be relatively susceptible to the peanut leafhopper.

**F. Screening for Tolerance to Low moisture Supply.** Twenty-five lines selected from the 1986 drought screening at IPB-UPLB based on pod and seed yield were planted at three locations (IPB, Pangasinan State University and MAF, Ilagan, Isabela) during the 1986-87 screening test. Based on consistent yield performance of each entry in three locations, six entries (Acc 847, 55-437, Pangasinan White, GNP 104, 47-16, IPB Pn-101) can be considered promising under drought conditions.

**G. Screening Peanut for Acid Tolerance.** Two hundred seven cultivars, advanced generation lines and accessions were evaluated for acid tolerance in five experiments in two soil types having high acidity. On Antipolo clay (Ultisol) with pH 4.1, exchangeable A1, 3.4 me/100 g and solution A1 6.8

ppm, the following entries produced the highest absolute and relative yields: IPB Pn 24-3, IPB Pn 24-2 and IPB Pn 24-6. Accession 25, which produced the highest yield on the limed plots (1.6 tons/ha), produced 0.77 ton/ha on the unlimed plots or a relative yield of 48%. The most promising selections from the advanced generation lines screened for acid tolerance were: IPB Pn 82-71-56, IPB Pn 82-71-26, IPB Pn 82-71-32 and UPL Pn-6.

On a Lipa clay loam (Alfisol) with a pH of 4.9 and exchangeable Al of 1 mc/100 g, the acid-tolerant entries after two screening experiments were BPI P9, UPL Pn-2 and RLRS 1. The top four entries from the advanced generation lines consist of IPB Pn 82-71-2, IPB Pn 82-71-29, IPB Pn 82-70-15 and IPB Pn 24-2.

Aluminum-tolerant entries selected from plots treated with 540 ppm Al were UPL Pn-4, IPB Pn 24-6, RLRS 13, PI 341108, PI 234375 and FDRS 19.

**H. Screening for Enhanced Nitrogen Fixation.** Sixty-four entries were screened for enhanced nitrogen fixation during the 1987 dry season. IPB Pn 49-12 ranked first followed by 57-422 in nitrogenase activity. IPB Pn 49-12 also had the highest dry matter yield and nodule dry weight.

The symbiotic performance of selected peanut entries under partial shade was evaluated during the 1987 wet season. Four peanut entries (UPL Pn-4, ICGS(E)-19, UPL Pn-2 and IPB Pn 3-127 M5) were tested against *Bradyrhizobium* strains 3G4b20, RP 182-13, P3 and CB 756. The entries were grown in clay pots containing Lipa clay loam-rice straw mixture and inoculated at planting. Phosphorus and potassium were applied in the forms of solophos and muriate of potash, respectively, at the rate of 50 kg/ha.

Plant samples were collected during the early pod-filling stage to assess nitrogenase activity, nodulation and dry matter yield. The different peanut entry-strain combinations showed reduction in nitrogenase activity under partial shade conditions. Reduction in nitrogenase activity ranged from 29-47%. On the other hand, nodule number and dry matter yield of some combinations were not affected by partial shading. However, total nodule mass and size of nodules were adversely affected by shading.

**I. Screening for Shade Tolerance.** The study started on October 1, 1987. It is a continuation of the peanut shade project which was previously funded by the N. C. Agricultural Research Service and which was terminated September 30, 1987. Confirmatory tests on shade-tolerant entries will be done using three shade levels (32 and 58% shading). Mass screening of entries under partial shaded conditions will be conducted during the 1987-88 dry season at the UPLB shade structure. Hybridization of shade-tolerant entries with high yielding upland lines are in progress.

## North Carolina

### A. Cultivar Development

**1. Evaluation of Advanced Breeding Lines.** The final evaluation of two advanced breeding lines, NC Ac 18411 and NC Ac 18423, in the Virginia-North Carolina Peanut Variety and Quality Evaluation Program (PQVE) was completed. NC Ac 18411, a selection from the cross of (Florigiant x NC 5) x (Florigiant x Valencia), and NC Ac 18423, a selection from the cross of (Florigiant x Florunner) x Early Bunch, both met minimum standards for release to North Carolina and Virginia peanut growers. Both lines have slightly higher yields than the check cultivar, Florigiant. Eighteen advanced breeding lines were selected and increased for evaluation in the PQVE program during 1988. Thirteen of the 18 lines were selected for yield and market grades. Of these 13 lines, eight came from the recurrent selection for yield program. Four lines were included for their resistance to *Cylindrocladium* black rot (CBR) and one because of its spider mite resistance. An additional 15 advanced breeding lines (5 with CBR resistance, 2 with insect resistance and 5 with high yields) were selected for seed increase during 1988 for inclusion in the 1989 PQVE. Sixty-four advanced breeding lines that outyielded the check Florigiant were selected for advanced testing in 1988. These lines include those selected for insect resistance, CBR resistance, leafspot resistance and early maturity. Twenty-six F<sub>7</sub> generation breeding lines from crosses of NC 17976/NC 9, NC 17921/NC

77-2, NC 77-5/NC 17922 and NC 77-6/NC 18222 outyielded check cultivars and were selected for further evaluation in 1988.

2. **Development of Breeding Lines.** Ninety-nine crosses in  $F_4$  generation from Cycle 3 of the recurrent selection program were seed increased for yield testing in 1988. High yielding crosses will be identified and individual breeding lines will be isolated from each cross for further evaluation. Approximately 225  $F_2$  families in  $F_4$  generation from the crosses of NC 9/NC 7 and its reciprocal and NC 7/Florigiant and its reciprocal were selected because of their large seed size. Individual lines will be selected from these families during 1988.

### 3. Selection for Disease Resistance

a. **Cylindrocladium black rot (*Cylindrocladium crotalariae*).** A breeding line designated NC Ac 18417 which resulted from the cross of NC 8C and Florigiant was released by the N. C. Agricultural Research Service as NC 10C. NC 10C has better seed quality and shelling characteristics than NC 8C but is slightly less resistant to CBR. Five lines (NC 17921<sup>3</sup>/NC 18229, NC 18230/NC 2, NC 18229/NC 2, NC 18016/NC 2 and NC 18230/NC 2) with good CBR resistance in 1987 and with high yields and good market grades are being increased for regional testing in 1989. Eighteen additional breeding lines were equal to the resistant check for CBR resistance during 1987. These 18 lines plus 19 selections from the cross of NC 18229A/NC 2 will be further evaluated in 1988. NC 9 and NC Ac 18411 were crossed to resistant parents NC 8C and NC 18016. The resulting hybrids will be increased and selection for resistance and agronomic traits will be practiced.

b. **Early leafspot (*Cercospora arachidicola*).** Seven breeding lines with early leafspot resistance and high yields were selected from crosses involving GP-NC 343. The seven lines do not have acceptable seed size and color. The seven lines were crossed in 1986 with NC 9, NC 17921, NC 18411 and NC 10C in order to improve seed characteristics. These crosses were advanced another generation during 1987. Selections will be made and evaluated for resistance and yield. Four additional sources of resistance (PI 109839, PI 270806, PI 269685, Kanyoma), all unadapted to North Carolina, were crossed with NC 6, NC 7 and Florigiant. Sixteen advanced breeding lines were selected for resistance during 1987. These lines are being tested in advanced leafspot and yield trials in 1988.

c. **Late leafspot (*Cercosporidium personatum*).** Three lines resistant to early leafspot (PI 109839, GP-NC 343, PI 270806) were crossed with three lines resistant to late leafspot (NC 17090, NC 17133R, FESR 5- P2-B1) to evaluate potential for combining both sources of resistance into single germplasm. Two crosses (NC 17090/PI 109839 and GP-NC 343/NC 17090) with reciprocals were selected based on combining ability and selfed to obtain  $F_2$  plants.  $F_2$  families were bifurcated in  $F_3$  and advanced to the  $F_3$  generation via single-seed descent. Fifty bifurcated  $F_3$  families from each cross will be evaluated for leafspot resistance and agronomic traits over two locations in 1988. Additive and additive x additive epistasis will be estimated for all traits.

Resistant genotypes (GP-NC 343, NC 17135) and cultivars adapted to different environments (NC 9, Lampang, Tainan 9, Chico) were crossed in a half diallel in 1986. Backcrosses were made and selfed generations to conduct a generation means analysis for resistance and agronomic traits. Eight generations ( $P_1$ ,  $P_2$ ,  $F_2$ ,  $F_3$ ,  $BC_{11}S_1$ ,  $BC_{12}S_1$ ,  $BC_{11}S_2$  and  $BC_{12}S_2$ ) from three crosses (GP-NC 343/Tainan 9, GP-NC 343/NC 17135 and Tainan 9/NC 17135) were used for testing additive, dominance and epistatic variance components. The additive-dominance model was sufficient to account for the variability in the cross GP-NC 343/Tainan 9 and Tainan 9/NC 17135 for all traits; however, epistasis in the form of additive x additive was important in the cross of GP-NC 343/NC 17135.

The diallel analysis was conducted at two locations in 1987. Additive variance was predominate but some nonadditive components were important for some of the disease components and most of the agronomic traits. Two different estimates of heritability were obtained with very different results. Seed will be sent to Thailand for evaluation for resistance and adaptability.

A 10-parent half diallel with parents resistant to late leafspot and rust (NC 17090, PI 341817, PI 405132, TG3/EC 76446), parents adapted to Thailand (Lampang, Tainan 9, Meket), a high yielding line (ICGS-4), an early maturing line [ICGS(E)-5] and a line with high nitrogen fixation (NC 2821)

was performed at ICRISAT. Combining ability analysis was conducted for early and late leafspot resistance in the  $F_1$  during 1985. The subsequent  $F_2$  generation was evaluated for resistance to virus and for yield in 1986. Material was evaluated for leafspot in the  $F_3$  generation at Khon Kaen, Thailand under natural conditions during the second growing season.

Additive effects were predominate for all traits. Differences in general combining ability effects were expressed in the  $F_1$  and  $F_2$  evaluations for leafspot resistance, percent peanut stripe virus infection and yield. The Virginia parents and progeny were less susceptible to peanut stripe virus. ICGS-4, PI 31817 and TG3/EC 76446 possessed the best general combining abilities for components of early and late leafspot resistance. Two crosses (PI 341817/(TG3/EC 76446) and PI 341817/ICGS-4) were selected for further study.  $F_2$  families were evaluated in the  $F_3$  generation for field resistance to early (Lewiston, NC) and late (Tifton, GA) leafspot resistance. Both high and low resistant selections were made. The selections will be tested in both locations in 1988 to obtain realized heritability estimates.

d. *Aspergillus parasiticus*. J11, Faizpur, Var. 27, Ah 7223, PI 337394F, PI 337409, Monir 240-30, UF 71513, U-4-47-7, C55-437, NC Ac 841, AR-1, AR-2, AR-3, AR-4, Sunbelt Runner, GFA-1 and GFA-2, all reported to be resistant to *Aspergillus* sp., were each crossed to Tainan 9, NC 7, SK 38 and NC 8C. Resistant lines crossed with NC 7 have been advanced to the  $F_4$  generation and  $F_5$  progeny will be evaluated for dry seed resistance to field infection and aflatoxin production in 1988.

To investigate the inheritance of three mechanisms of aflatoxin resistance, a crossing program is being conducted to obtain six generations for a generation means analysis. Adapted parents (NC 7 and Gadjah from Indonesia) will be crossed with a line with reported seed-coat resistance (J11) and a line with resistance to aflatoxin production (U 4-7-5) in 1988. The genetic relationship among the resistance mechanisms will be investigated.

e. *Sclerotinia blight* (*Sclerotinia minor*). Sixty-two advanced breeding lines were provided to Virginia cooperators for evaluation of *Sclerotinia blight* resistance during 1986. The 62 lines included 27 backcross lines from the cross of (Florigiant/Florunner)<sup>3</sup>/Chico, 33 backcross lines from the cross of (Florigiant/Florunner)<sup>3</sup>/GP-NC 343, and 2 lines from the cross of NC 18016A/NC 2. Several of the lines showed promise with one line from the Chico cross being the most resistant of the lines evaluated in this study. Lines from these crosses plus an additional 15 lines of the (Florigiant/Florunner)<sup>3</sup>/Chico cross were evaluated in 1987. Because of environmental conditions during 1987, the data on *Sclerotinia blight* resistance did not distinguish among the resistant genotypes. Forty-six of the lines from these crosses will be evaluated for *Sclerotinia blight* resistance in Virginia during 1988.

NC 7, NC 18411 (FG/NC 5//FG/Val) and NC 10C were crossed as females with four sources of resistance (three from O. Smith, TX and one from P. Phipps, VA) to *Sclerotinia blight* (TX 498731, TX 798736, TX 804475 and TRC 02056-1) during 1986.  $F_2$  plants from these 12 crosses were grown in Lewiston during 1987. Several  $F_2$  crosses were selected on pod and seed characteristics and their  $F_3$  progeny are being screened for resistance to *Sclerotinia blight* during 1988. In addition, a single seed descent population from each cross was developed and will be increased at Lewiston during 1988.

Seven plant introductions (PIs 467829, 476831, 476834, 476835, 476842, 476843 and 476844) from China identified as resistant to *S. minor* by Porter et al. were crossed to NC 9, NC 18411, NC 18417 and Sunrunner in early 1987. The  $F_1$  plants were grown in Puerto Rico in 1987-88. Progenies from these crosses are being grown in Lewiston in 1988. Single plant selections will be made for evaluation to *Sclerotinia blight* in 1989.

#### 4. Selection for Insect Resistance

a. *Potato leafhopper* (*Empoasca fabae*). Breeding lines resistant to southern corn rootworm also possess moderate resistance to potato leafhopper. These sources of resistance have been used in crosses and sent overseas for testing. GP-NC 343 has been used as a donor parent in a multiple backcrossing program with breeding line NC 17921. Twenty-seven  $B_1C_2$  lines were evaluated for

yield and insect resistance in the  $B_1C_2F_4$  and  $B_1C_2F_3$  generations. Two lines are being increased for evaluation in the Virginia-North Carolina Variety and Quality Evaluation Program in 1989.

Crosses of NC 7 with leafhopper-resistant lines (GP-NC 343, NC 302, NC 15745, NC 10247, NC 10272 and NC 301) were made and increased to  $F_4$ . Individual plant selections on seed characteristics were made in 1987 and will be tested in  $F_5$  for leafhopper resistance in 1988.

b. **Two-spotted spider mite (*Tetranychus urticae*).** Three breeding lines—GK 53, GP-NC 343 and NC 17367 (Florigiant x Florunner)— were found to have moderate resistance to spider mite. All three lines were crossed to several breeding lines and selection for yield and resistance was practiced. One line [NC 18426 (NC 17367/NC 7)] has shown some promise in two years of testing in the Virginia-North Carolina Variety and Evaluation Program. One other line (GP-NC 343/GK 3) will be included in the regional trials next year.

5. **Heritability.** The  $F_3$  generation of a selected cross (NC 6/ICG 11) was grown during 1987. The heritability of  $N_2$ -limiting characteristics in the  $F_4$  generation of the cross will be estimated by evaluating 55  $F_{2,4}$  families at two locations in 1988. Material from the same cross that was bifurcated in the  $F_2$  is being increased to the  $F_3$  for estimation of additive and additive x additive genetic variance. High and low acetylene reduction selections from the BNF recurrent selection program are being advanced to inbred lines and evaluated for acetylene reduction and yield.

#### 6. Breeding for Early Maturity, Seed Storage, Dormancy and Large Seed

a. **Combining ability for early maturity and seed dormancy.** A half diallel crossing program was performed using five spanish lines and three virginia lines. The five spanish lines possessed early maturity and the three virginia lines possessed dormancy. From  $F_1$  data the GCA effects of parameters measured for maturity and dormancy in both greenhouse and field were significant. Chico produced the earliest maturing progeny. NC 7 and 73-30 produced progeny with the best dormancy.

A crossing program was conducted in 1988 to study the inheritance of early maturity. The parents are: No. 334 (adapted to Pakistan), Banki (adapted to Pakistan), NC 9 (adapted to U.S.), ICGS(E)-4 (early maturity from ICRISAT), ICGS(E)-136 (early maturity from ICRISAT), ICGS(E)-147 (early maturity from ICRISAT) and Chico (early maturity line). The crosses NC 7/Chico and 73-30/Chico were selected for further evaluation. The  $F_2$  generation was evaluated in 1987 and 1988, 120  $F_2$  families in  $F_3$  generation from each cross will be evaluated for maturity and dormancy in a replicated field trial.

b. **Heritability for early maturity and fatty acid composition.** Two crosses from an eight-parent diallel previously evaluated for fatty acid composition were selected for a heritability study. Narrow-sense heritabilities were calculated for crosses, NC 7/NC 9 and NC 7/55-437 using parent-offspring regression of  $F_{2,3}$  means on  $F_2$  plants. Narrow-sense heritabilities were low to medium for fruit size, early maturity and O/L ratio. In cross 1, NC 7/NC 9, several  $F_{2,3}$  families exhibited large fruit length, early maturity and higher O/L ratios in which narrow-sense heritabilities are 0.47, 0.38 and 0.51 for these traits, respectively. This material is presently being grown for selection in the  $F_4$  generation.

c. **Gene action estimation for early maturity and fatty acid composition.** A generation means analysis was used to estimate the genetic parameters in an additive-dominance model for two crosses, NC 18411/Chico and NC 18411/55-437, for early maturity and fatty acid composition. A three-parameter model fit the observed data well, implying little epistatic influence on the inheritance of these traits. Since the additive component [a] was consistently significant and the dominance component [d] was not different from zero, selection in early generations for these two populations may be possible.

Sixty crosses made during 1985 in the  $F_2$  generation were generated by crossing 20 lines selected for early maturity and high O/L ratios with three adapted virginia lines in an  $M \times N$  mating design. The  $F_2$  and the  $F_3$  generations were evaluated for combining ability. GCA estimates in both generations were significant and greater in magnitude than SCA estimates. There were no  $F_2$  progeny that

had both earliness and high O/L ratios; however, in the  $F_3$  generation, some families had high yield and higher O/L ratios but none that had high yield, early maturity and higher O/L ratios.

d. **Genetics of large seed.**  $F_1$  hybrids and parents from a partial diallel of 50 exotic and adapted peanut lines will be planted in a nursery in 1988. Pod and seed size characteristics will be recorded, material will be advanced and studied further. Some of the hybrid material was advanced in Puerto Rico and the subsequent  $F_2$  seed were planted in 1988. Promising crosses will be selected and plants harvested for studying the heritabilities of large seed. Selections will be made based on seed size and agronomic appearance.

## B. Breeding Methodology and Genetic Studies

1. **Recurrent Selection for Yield—Elite Virginia Germplasm.** Three cycles of recurrent selection for yield for a population generated by making random crosses among 40 elite virginia-type peanut lines have been completed. The recurrent procedure provides for a large number of crosses among diverse genotypes, minimizes the number of pollinations per cycle of recombination, allows testing in the  $S_3$  generation, and under ideal conditions, requires only 2 years per cycle. The parental lines for each of the three cycles were used to determine progress from selection and genetic variance in the population.

The fourth cycle of recurrent selection is presently in replicated yield trials at two locations in North Carolina. The top 40% of the lines will be selected and randomly intermated in the greenhouse to produce 100 crosses (each of the 40 lines used as a parent in five crosses) which will initiate the fifth cycle of recurrent selection. In addition, the highest yielding lines (top 10%) from the 1988 yield trial will be space planted in 1989 and individual plant selections will be made. These selections will subsequently be used to generate progeny rows, selections from which will go into regional yield trials.

2. **Effectiveness of Convergent Crossing.** A convergent crossing program was initiated in 1986. Fourteen topcrosses with a potential cultivar (NC 18411) were performed. The lines used in the topcrosses were selected for their various traits: early maturity (Pronto, Va 81B, Chico), drought resistance (57-422, NC 17090, 59-127), CBR resistance (NC 18229, NC 10C, NC 8C, Tifton 8) and leafspot resistance (GP-NC 343, GP-NC 343/NC 5, Florigiant/GP-NC 343, FESR-11-P11-32). The first stage of the convergent cross was performed in 1986. The 14  $F_1$ s generated in 1986 were selfed in the greenhouse to produce  $F_2$ s. The  $F_2$ s were planted in replicated yield trials at two locations in North Carolina in 1987. Based on yield and agronomic characters (short market grades) in the  $F_2$ , reserved seed (in the  $F_1$  generation) of selected lines were used in the second stage of the convergent cross.

3. **Establishment of Isozyme Patterns.** Different tissues of 4 spanish, 4 valencia and 5 virginia-type peanut cultivars are being characterized for banding patterns of various isozyme systems using starch gel electrophoresis. The progeny of crosses between the botanical types ( $F_1$  and  $F_2$ ) will be included in the study to determine the inheritance of isozyme differences.

## C. Cytogenetic and In vitro Studies

1. **Interspecific Hybridization.** Recently introduced diploid species accessions from South America were hybridized with *A. duranensis* (A genome) and *A. batizocoi* (B genome) species to identify genomic groups and potentials for gene incorporation into the cultivated peanut. Plants from 68 interspecific hybrid combinations were propagated in the greenhouse and fertility data collected to identify hybrids. Meiotic chromosomes were analyzed in more than 100 plants of 56 hybrid combinations. Most species accessions were found to have an A genome. Four B genome accessions of *A. batizocoi* were analyzed and cytological variation was observed among accessions. A standard karyotype was described for this species. A third genome was identified in section *Arachis* and several other accessions were sterile when hybridized with both *A. duranensis* and *A. batizocoi*. In addition to crosses with the two diploid genome testers, hybrids with the cultivated peanut were also made. As a result of the investigation, a more complete synthesis of the biosystematic relationships with species closely related to the cultivated peanut will be forthcoming along with potentials for introgression of desired traits from the *Arachis* species to *A. hypogaea*.

Hexaploid interspecific hybrids between *A. hypogaea* and diploid species of section *Arachis* were propagated in the field to increase generation numbers and to increase opportunities for genetic recombination. Eleven different hexaploid combinations were used in crossing programs with *A. hypogaea* to lower the chromosome number to that of the cultivated peanut. In addition, pentaploid interspecific hybrids were identified and propagated with six different species in their pedigrees. Cytological observations indicated that many of these produce offspring with lower chromosome numbers ranging from  $2n = 40$  to 44.

**2. Embryo Rescue.** Embryo culture experiments were completed which identified growth regulator combinations useful for supporting peanut tissues *in vitro* and enhancing growth. Embryos of *A. hypogaea* can now be routinely grown to maturity. Crossing programs were conducted to produce interspecific hybrid embryos of incompatible species to obtain reproductive tissues for applying embryo rescue techniques. Of 124 embryos cultured from pollinations between hexaploid x diploid species, two new interspecific hybrids were obtained. Although the success rate was low, techniques show the feasibility of obtaining otherwise incompatible hybrids by *in vitro* techniques.

Because embryos of many desirable interspecific hybrids in the genus abort prior to the developmental stage where embryos can be rescued, peg tips of 1- to 4-day-old tissues were cultured *in vitro*. Embryo growth was observed in many of the 25 media combinations used with concentrations of two auxins and two cytokinins. Kinetin was more beneficial than benzylaminopurine for enhancing early embryonic development. One heart-shaped embryo was observed in histologically observed sections from tissues grown on a kinetin-indoleacetic acid media. Experiments are being conducted to confirm experiments which induced both growth and differentiation of embryos.

Embryo growth of 1-4-day-old tissues has also been observed in culture media without growth regulators. Thus, *in vitro* experiments were conducted with nine basal media to test their effects on embryo growth to establish optimal growing conditions for reproductive tissues. Three-day-old embryos were collected and placed on media and after 21 days they were preserved in a fixing solution and embedded in paraffin for histological analyses.

**3. In vitro Regeneration.** Attempts were made to duplicate successes with other legumes for plant regeneration. Experiments were conducted with 15 genotypes, 2 explants and 3 different media. Significant variation was observed for *in vitro* responses, but mature plants were not regenerated. Additional tests to induce roots on peanut tissues were then conducted. Four genotypes representing previously tested cultivars for good and poor responses to *in vitro* experiments were used in experiments with 50 media combinations of four growth regulators. Preliminary results show significant differences among genotypes and media for root responses.

**4. Anther Culture.** Attempts are being made to develop anther culture techniques to reduce efforts required to form pure lines. Haploid plants could also be more adaptable with diploid wild species (with lower ploidy number). Work was done on staining, staging and photographing peanut microspores from meiosis through binucleate stages. Anthers were cultured in four different liquid basal media and one liquid media with growth regulators for 4, 7 and 10 days; the microspores were observed for viability and divisions. Anthers were also cultured for 7 days on 23 different semi-solid agar or agarose media with or without growth regulators and assessed in the same manner. N6 medium solidified with agarose with NAA, kinetin and high glutamine was found to be most suitable for microspore responses. The most suitable sucrose and glutamine concentrations will be elucidated as well as an analog of glutamine in place of glutamine. Microspore divisions up to four nuclei have been observed most frequently in anthers which were cultured at the early uninucleate stage.

#### D. Disease Resistance Research

**1. Cylindrocladium Black Rot (CBR).** Although CBR is currently a serious disease problem on peanut in the USA and Australia, the pathogen has the potential to cause losses in legume crops (peanut, soybean, forages, etc.) throughout the cooler agricultural areas of the world. This fungus, as well as several closely related species, have international distribution and probably were introduced (CBR) into the USA only a few decades ago.



Biological studies are necessary for development of management strategies for CBR of peanut as well as similar pathogens on diverse crops. Epidemiological studies were conducted utilizing seven peanut genotypes in two cropping rotations (corn, peanut) in North Carolina fields which have natural populations of northern (*Meloidogyne hapla*) and peanut (*M. arenaria*) root-knot nematodes and CBR inoculum. These studies are being used to determine relations between nematode populations and fungus inoculum density, soil fertility factors, and disease progress of CBR in resistant and susceptible peanut genotypes. Similar tests were conducted in field microplots and greenhouse environments using low, moderate and high levels of fungal inoculum and two levels of nematode inoculum. Both nematode species enhanced root knot severity in all genotypes. However, *M. arenaria* had a greater effect on root rot severity in moderately resistant genotypes than did *M. hapla*. Results of these tests indicated that resistance in certain genotypes react differently when in combination with nematode damage than others, i.e., expression of resistance was not drastically reduced by these nematodes. Further information from these different reactions should be valuable for both CBR management and the breeding program.

Preliminary studies on resistance in peanut to root-knot nematodes suggests that progress could be made in breeding new peanut cultivars. Investigations of the possibilities for use of newly identified sources of resistance to root-knot nematodes should provide insight that would complement on-going programs for control of root diseases as well as for the nematodes themselves.

Basic epidemiology studies continue utilizing fields naturally infested with *Cylindrocladium* black rot to determine spatial and temporal relationships of CBR on resistant and susceptible peanut genotypes. Nine peanut fields were sampled for microsclerotia of *Cylindrocladium crotalariae* and for incidence of CBR. Three fields each of NC 8C, NC 10C and Florigiant were selected. The data will be analyzed to determine if patterns of inoculum in soil are correlated with patterns of disease incidence and to determine if moderate resistance in NC 8C or NC 10C affects any correlations found.

2. *Cercospora* Leafspot. Virulence of a North Carolina (NC) and a Thai (T) isolate of *Cercosporidium personatum* was compared on peanut genotypes with low to high partial resistance to the pathogen. Leaves were inoculated with either isolate and incubated 6 days at 26/18, 30/22 or 34/26 C (day/night). Both isolates produced fewer lesions on all genotypes as temperature increased, but isolate T always caused more infections than NC. Lesion number was correlated with spore germination by each isolate. Other leaves were inoculated, incubated 6 days at 20 C and transferred to 26/18, 30/22 or 34/26 C. Post-infection development of both isolates on genotypes with low, moderate or high resistance was similar at all temperatures. Although fewer genotypes sporulated at higher temperature, all genotypes had sporulating lesions at 34/22. Lesions of T produced fewer spores than NC at all temperatures.

The time required for infection of five peanut genotypes also was compared for the two isolates of *C. personatum*. Detached leaves of each genotype were inoculated individually with each isolate and misted for 1 to 8 days after inoculation. The Thai isolate was insensitive to increases in misting period, but the US isolate caused more lesions when mist periods were longer. The US isolate caused more infections than the Thai isolate on all genotypes and for all mist periods.

Six peanut genotypes were planted in isolation plots surrounded by corn in spring 1988. The genotypes had various levels of partial resistance to *Cercospora arachidicola* or *C. personatum* or both pathogens. Plots were provided with a source of inoculum of either pathogen, or both, or were not exposed to any inoculum in addition to that present naturally. Leafspot epidemics were monitored in all plots. The results will show if shifts in the relative proportion of each leafspot pathogen occur in response to deployment of resistance to one or both pathogens.

Studies are continuing on the ecology of pathogen survival in lesion areas of decayed peanut leaflets. The role of antimicrobial compounds produced by *Cercospora* spp. is being determined by burial of leaflets in field sites and subsequent retrieval of leaflets and incubation in the laboratory. Shallow burial of leaflets enhances survival of *C. arachidicola* during winter seasons in North Carolina.

3. *Sclerotium rolfsii* and *Sclerotinia minor*. Nurseries were established in 1988 for screening peanut for resistance to *S. rolfsii* and *S. minor*. Separate nurseries for each pathogen were established

in adjacent areas. One-half of each nursery was used for resistance screening in 1988. The remaining half of each nursery was planted with a susceptible cultivar and inoculated with the appropriate pathogen. Use of alternate halves of each nursery in alternate years is intended to increase uniformity of inoculum within fields and, therefore, increase accuracy and precision of screening results.

Microplots established in 1988 in two locations were infested with either *Meloidogyne arenaria* or *M. hapla* and were planted with four peanut genotypes. The microplots were infested with sclerotia of *S. rolfsii* to determine if root-knot nematodes affect stem rot incidence or severity on the test genotypes. Slightly less disease developed overall on plants inoculated with *M. hapla*, compared with plants inoculated with *M. arenaria* or uninoculated plants. NC 2 had fewer stem rot lesions than the other genotypes, especially in plots infested with *M. arenaria* at both locations.

Three peanut genotypes with differing canopy morphologies were planted in 1987 in microplots infested with *S. rolfsii* and were sprayed with insecticides and fungicides as needed to prevent defoliation, or were not sprayed. Sprayed or unsprayed plots either were covered with a shelter that excluded rainfall, were covered and irrigated, or were not covered. Disease incidence on all genotypes was least in covered, nonirrigated plots, and disease incidence on Florigiant and NC 2 was greatest in uncovered plots. NC Ac 18016, which has a compact canopy, was most diseased in covered, watered plots. Disease incidence was slightly greater on the average on plants that were not sprayed than on plants that were sprayed. The experiment was re-established in 1988.

**4. Peanut Stripe Virus (PSV).** A dot-blot immunobinding assay and three variants of ELISA; the direct double antibody sandwich (DAS-ELISA), Protein-A ELISA, and an indirect ELISA were evaluated for detecting peanut stripe virus in crude and clarified sap preparations from peanut leaves. Whole IgG was used with both DAS-ELISA and Protein-A ELISA. The indirect ELISA and the dot-blot assay were carried out using fragments of immunoglobulin. Alkaline phosphatase Protein-A conjugate was used for the Protein-A and the indirect ELISA, while alkaline phosphatase-goat anti-rabbit conjugate was utilized for the dot-blot assay. Optimization experiments were conducted for each method. Three ELISA methods performed well for detection of PSV and choice of method to use largely depends on the purpose and availability of antisera and equipment for further purification and fractionation of antisera. The indirect ELISA gave significantly lower healthy control values than the other two variants. However, there were no significant differences among levels from infected leaves. The dot-blot assay was more sensitive than the three ELISA methods; however, indirect ELISA using fragments appeared to be more suitable for quantitative assay.

Peanut stripe virus accumulation and symptom development was determined for eight peanut genotypes representing three botanical types: valencia, virginia, and spanish. Using an indirect ELISA, the relative amount of virus was monitored weekly for 10 weeks after inoculation on leaves at different nodal position. Symptoms varied within the plant canopy; symptom types followed a sequential pattern on all genotypes. Initial symptoms (mild mottle) occurred 9-14 days after inoculation on the fifth leaf of the main stem and third leaf of the lateral branch. Yellow mosaic, dark green patches and stripes were observed on newly developed leaves 14-26 days after inoculation. A mild oak leaf pattern symptom was observed on developing leaves approximately 28 days after inoculation. Severity of symptoms was correlated with virus titer levels on all genotypes. Virus titer was highest on leaves showing yellow mosaic and dark green patches during the second and third week after inoculation. Virus titer then declined at the fourth and succeeding weeks after inoculation. Systematically infected leaves attained higher virus titer levels than inoculated leaves. Differences in virus titer was observed between botanical types during the second, third and fourth week after inoculation on both the inoculated leaf and systematically infected leaves. Except for one cultivar, no difference in virus titer was observed within genotypes of the same botanical type. Spanish and valencia-type genotypes had higher virus titer than the virginia-type genotypes. Florigiant had higher virus titer than the two other virginia-type cultivars, NC 6 and NC 7.

## TRAINING OUTPUTS

Surname	Sex	Univ.	Dept.	Degree	Date degree rec'd.	CRSP support
<u>U.S. citizens</u>						
Anderson	M	NCSU	Crop Science	PhD	—	Total
Rachmeler	M	NCSU	Crop Science	PhD	1988	Total
Fitzner	M	NCSU	Crop Science	PhD	—	Partial
Arrendell	F	NCSU	Crop Science	PhD	1987	Partial
Mercer	F	NCSU	Crop Science	MS	1988	Partial
Halward	F	NCSU	Crop Science	PhD	—	Partial
Reece	F	NCSU	Crop Science	MS	1988	Partial
<u>Thai citizens</u>						
Jogloy	M	NCSU	Crop Science	PhD	1988	Total
Charoenrath	M	NCSU	Crop Science	PhD	—	Total
<u>Filipino citizen</u>						
Aquino	M	NCSU	Plant Pathology	MS	1987	Total
<u>German citizen</u>						
Grieshammer	F	NCSU	Crop Science	MS	—	Total

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## PUBLICATIONS

### Theses

- Aquino, V. M. 1987. Biology of peanut stripe virus (PStV) on *Arachis hypogaea* L. M.S. Thesis, NCSU (Director: M. K. Beute).
- Arrendell, S. 1987. Breeding for increased biological nitrogen fixation of peanut (*Arachis hypogaea* L.). Ph.D. Thesis, NCSU (Director: J. C. Wynne).
- Fore, S. A. 1987. The role of cercosporin in peanut leaf spot. M.S. Thesis, NCSU (Director: M. K. Beute).
- Jogloy, S. 1988. Estimate of gene action on leafspot resistance and agronomic traits in peanut (*Arachis hypogaea* L.). Ph.D. Thesis, NCSU (Director: J. C. Wynne).
- Mekontchou, T. 1987. Inheritance and combining ability for early maturity and seed dormancy for a selected group of peanut lines (*Arachis hypogaea* L.). M.S. Thesis, NCSU (Director: J. C. Wynne).
- Mercer, L. C. 1988. Inheritance of fatty acid content in peanut (*Arachis hypogaea* L.). M.S. Thesis, NCSU (Director: J. C. Wynne).
- Rachmeler, D. N. 1988. Inheritance of early maturity and fatty acid composition in peanut (*Arachis hypogaea* L.). Ph.D. Thesis, NCSU (Director: J. C. Wynne).
- Reece, P. 1988. Effects of four growth regulators on the *in vitro* culture of peanut embryos. M.S. Thesis, NCSU (Director: H. T. Stalker).

### Manuscripts

- Anderson, W. F., J. C. Wynne and M. K. Beute. 1986. Combining ability and heritability of resistance to early and late leafspot of peanut. Peanut Sci. 13:10-14.
- Fore, S. A., M. E. Daub and M. K. Beute. 1987. Phytotoxic substances produced by some isolates of *Cercospora arachidicola* are not cercosporin. Phytopathology (accepted).
- Jogloy, S., J. C. Wynne and M. K. Beute. 1987. Inheritance of late leafspot resistance and agronomic traits in peanut. Peanut Sci. 14:86-90.
- Moss, J. P. and H. T. Stalker. 1987. Embryo rescue in wide crosses in *Arachis*. 3. *In vitro* culture of peg tips of *A. hypogaea* selfs and interspecific hybrids. Peanut Sci. 15:70-74.
- Shew, B. B., T. Sommartya and M. K. Beute. 1987. Components of partial resistance in peanut genotypes to isolates of *Cercosporidium personatum* from the United States and Thailand. Phytopathology (accepted).
- Arrendell, S., J. C. Wynne, G. H. Elkan and T. J. Schneeweis. 1988. Selection among early generation peanut progeny for high and low acetylene reduction and plant weight. Crop Sci. (in press).
- Monteverde-Penso, E. and J. C. Wynne. 1988. Evaluation of three cycles of recurrent selection for fruit yield within a population of virginia-type peanut. Crop Sci. 28:75-78.

- Moss, J. P., H. T. Stalker and H. E. Pattee. 1988. Embryo rescue in wide crosses in *Arachis*. 1. Culture of ovules in peg tips of *A. hypogaea*. *Ann. Bot.* 61:1-7.
- Pattee, H. E., H. T. Stalker and J. P. Moss. 1988. Embryo rescue in wide crosses in *Arachis*. 2. Embryo development in cultured peg tips of *A. hypogaea*. *Ann. Bot.* 61:103-112.
- Shew, B. B., M. K. Beute and J. C. Wynne. 1988. Effects of temperature and relative humidity on expression of resistance to *Cercosporidium personatum* in peanut. *Phytopathology* 78:493-498.
- Alwi, N., J. C. Wynne, J. O. Rawlings, T. J. Schneeweis and G. H. Elkan. 1989. Symbiotic relationship between *Bradyrhizobium* strains and peanut (*Arachis hypogaea*). *Crop Sci.* 29: In press.
- Arrendell, S., J. C. Wynne, G. H. Elkan and T. J. Schneeweis. n.d. A lesson in breeding for enhanced nitrogen fixation in peanut. *Peanut Sci.* (submitted).
- Arrendell, S., J. C. Wynne, G. H. Elkan and T. J. Schneeweis. n.d. Selection among early generation peanut progeny for high and low acetylene reduction and plant weight. *Plant Breed.* (submitted).
- Arrendell, S., J. C. Wynne and J. O. Rawlings. n.d. Genetic variability and selection for acetylene reduction in peanut. *Crop Sci.* (submitted).
- Jogloy, S., J. C. Wynne and M. K. Beute. n.d. Diallel analyses for early and late leafspot resistance and agronomic traits in peanut (*Arachis hypogaea* L.). *Peanut Sci.* (in review).
- Jogloy, S., J. C. Wynne and M. K. Beute. n.d. Generation means analyses for leafspot resistance and agronomic traits of peanut (*Arachis hypogaea* L.). *Peanut Sci.* (in review).
- Stalker, H. T. and M. A. Eweda. n.d. Ovule and embryo culture of *A. hypogaea* and interspecific hybrids. *Peanut Sci.* (submitted).

#### PLANS FOR 1988-89

The research outlined in detail on pages 229-234 of the 1985 annual report will be continued. Emphasis in both Thailand and the Philippines will be to hybridize adapted germplasm with desirable sources of resistance to diseases and insects. The coordinated breeding-pathology program will continue in Thailand. Objectives in the Filipino program will be focused to concentrate on disease and insect resistance. Less emphasis will be given to acid-tolerance screening and to selection for increased nitrogen fixation. Studies at NCSU will focus on providing techniques/information and germplasm that will support the breeding efforts in Thailand and the Philippines. Efforts to extend the information and germplasm to small growers in Thailand and the Philippines will be increased.

TX/MM/S

### **Mycotoxin Management in Peanut by Prevention of Contamination and Monitoring**

Texas A&M University—Institut Senegalais de Recherches  
Agricoles—Institute de Technologie alimentaire  
Robert E. Pettit, Principal Investigator, TAMU  
Amadou Ba, Principal Investigator, ISRA—Amadou Kane,  
Principal Investigator, ITA

#### **INTRODUCTION**

Mycotoxin contamination of peanut poses a serious health hazard to consumers of peanut products throughout the world. The mycotoxin management project is designed to conduct experiments which can help discover improved procedures for reducing mycotoxin problems through prevention of contamination by development of new improved production practices and resistant varieties, monitoring of peanuts in trade channels for diversion of contaminated lots into either clean up or detoxification, the development of rapid, practical, and economical methods for the detection of mycotoxins in peanuts and peanut-derived products, and to discover safe and effective detoxification protocols for use in peanut oil and peanut-derived products.

During the past year, major efforts have concentrated on field screening peanut cultivars for possible resistance to invasion by the aspergilli; field plot studies to determine the influence of gypsum application on *Aspergillus* activity; studies to determine the importance of peg invasion; characterization of potential biochemical resistance mechanisms within host plant tissues; verification of the accuracy of the Selectively Absorbed Mycotoxin (SAM) assay procedure to detect aflatoxin in kernels of various peanut cultivars; and efficacy of attapuigite (from West Africa) as an sorbent of aflatoxin in crude peanut oil. The discovery of mycotoxin management strategies by researchers in Senegal and Texas should have application throughout the world wherever contamination of peanut and peanut-derived products is a problem.

#### **MAJOR ACCOMPLISHMENTS**

##### **Training**

Mr. Ahmedoul Bashir Sarr, graduate student from Senegal, has completed his second year of study and research program towards obtaining a Ph.D. degree within the Department of Veterinary Public Health at Texas A&M University. He has gained additional experience on the use of high pressure liquid chromatographic (HPLC) assay, enzyme-linked immunosorbent assay (ELISA), and selective absorption of mycotoxins (SAM) procedures for mycotoxin analysis. He has also tested several research procedures which will be helpful in verifying the safety and effectiveness of detoxification protocols.

Mr. Julius E. Fajardo, graduate student from the Phillipines, has completed his first year of course work and initiated his research program, a part of the program associated with a Ph.D. degree within the Department of Plant Pathology and Microbiology at Texas A&M University. Julius has gained experience on use of the high pressure liquid chromatograph to separate various phenolic acids from peanut plant parts and on the use of a Phast electrophoretic system for separation of proteins from peanut kernels using the highly sensitive silver-staining technique.

##### **Research Results**

The incidence of *Aspergillus flavus* and *A. niger* in soil from field plots near Waller, Texas revealed a gradual buildup of viable propagules from mean levels of 26 propagules per gram of soil in June to over 2,000 propagules per gram of soil in September and October.

The level of *A. flavus* in soil from field plots near Niore, Senegal treated with gypsum applications revealed that the lowest recovery rates at harvest occurred in soil samples from plots treated with gypsum 30, 45, and 60 days after sowing.

Field screening 20 peanut cultivars for tolerance to invasion by *A. flavus* by an examination of the pegs, pods, and kernels revealed that plant parts from the cultivar J-11 contained a mean infestation level of 2.9% compared to a level of 6.7% for the cultivar Florunner. A selection from a cross of Toalson/PI 365553/Tamnut-74 (B 815667) and a selection from the cross Toalson/UF 73-4022 (B804472) had mean infestation levels of 3.2% and 3.9% respectively.

Under field conditions peg infestation levels ranged from 0 to 7% with mean infestation levels of 0% for J-11, 5% for plants from a cross of Toalson/UF 73-4022 (TX 798736) and 6% for plants from the cultivar Florunner.

Electrophoretic analysis of sound peanut cotyledons from 14 cultivars for their protein patterns revealed the presence of 253 components with molecular weights which ranged from 14 to 520 KDa and with isoelectric points which ranged from pI 3.0-7.8. Differences in protein patterns among cultivars were noted.

Studies on changes of protein patterns, within live peanut kernels, as induced by *A. flavus* and *A. parasiticus* revealed the presence of pathogenesis-related proteins (PR) 13-24 hrs after inoculation. These PRs occurred in a basic region of 2-D electrophoretic gels and these patterns differed among cultivars.

Electrophoretic studies on proteins induced by *A. flavus* and *A. parasiticus* in peanut kernels from plants grown under drought stress (DS) and under normal irrigations (NI) revealed that kernels from DS plants lacked PRs except in the case of kernels from a selection of the cross Toalson/UF 73-4022 (TX 798736). Kernels from all cultivars tested and grown under NI contained PRs.

High pressure liquid chromatographic (HPLC) analysis of peanut seed coats and pods from 12 cultivars for various phenolic acids (compared with 18 standard compounds) has revealed the presence of similar phenolic acids at different concentrations within plant parts of different ages and within tissues of different peanut cultivars. Immature pods, mature pods, and mature testae contained the highest concentrations of phenolic acids.

Aflatoxin analysis of food products in the markets of Dakar, Kaolack, and Diourbel, Senegal revealed that 29% of the peanut samples contained aflatoxin with a mean level of 230 parts per billion (ppb); 60% of the processed peanut products (e.g. peanut paste and chocolate peanut butter) contained aflatoxin with mean levels of 42 ppb; and 50% of the corn samples tested contained aflatoxin with mean levels of 84 ppb.

Cooking aflatoxin contaminated peanut paste (butter) for 1-2 hrs in water reduced the aflatoxin content of the peanut butter by 73-85% of the original concentration.

Samples of crude peanut oil from Touba Toul, Senegal, with 60 ng/ml of aflatoxin B1, when treated with a local Senegalese clay (ground attapulgite) revealed that over 90% of the aflatoxin was bound by this clay. Procedures for removal of the fine clay particles from the oil without specialized equipment is in progress.

A simple multi-mycotoxin assay for the simultaneous detection of aflatoxin and zearalenone has been developed. The assay procedure has also proven to be user-friendly, inexpensive, chemically stable, can be held without refrigeration, and is capable of accurately detecting aflatoxin (compared to detection by HPLC) in peanuts from Niger.

#### EXPECTED IMPACT OF PROJECT

**In Senegal**—Reducing the levels of aflatoxin in Senegalese grown peanuts will improve the health of the local population and improve the quality of peanut meal produced. New methods of aflatoxin detection should aid in the diversion of mycotoxin contaminated peanuts into processing for clean up

and/or detoxification. Development of marketing procedures which provide an incentive for producing aflatoxin-free peanuts will help encourage implementation of preventive measures for reducing mold damage in the field and in storage. Newly developed aflatoxin sorption methodologies will allow local villagers to treat peanut oil to reduce aflatoxin levels to a safe level for consumption. Future development of peanut cultivars with resistance to penetration by the mycotoxin producing fungi will further reduce the chances of contamination.

**In United States**—Research results from efforts on this project will lessen the impact of the aflatoxin problem to the peanut industry in the U.S. Newly developed aflatoxin resistant peanut cultivars adapted to the peanut growing regions of the United States could greatly reduce the number of segregation III peanuts marketed within the country. Improved detection of aflatoxin contaminated peanuts and improved chemical assay techniques should increase the speed and accuracy of analyses and reduce costs related to the diversion of contaminated peanut lots. Discoveries related to the diversion and detoxification of aflatoxin in peanuts, peanut products, and other commodities will lessen the potential health hazards that contaminated products currently impose on the American public.

## GOAL

The goals of the Peanut CRSP mycotoxin management research project are to develop and apply techniques which reduce the severity of the mycotoxin contamination problems within the LDC's and the United States through prevention of contamination in foods and feeds, development of improved inspection and diversion procedures, and the discovery of cleanup and detoxification procedures which will render contaminated products safe for consumption.

## OBJECTIVES

- A. To determine the incidence of *Aspergillus flavus* in peanut field soils as influenced by production practices.
- B. To screen peanut cultivars under field conditions for tolerance (resistance) to invasion by the aflatoxin producing fungi.
- C. To determine protein patterns of sound peanut kernels from different cultivars and relate these patterns to susceptibility to aflatoxin contamination.
- D. Determine the relationship of pathogenesis related proteins (as a possible defense reaction of live peanut kernels) to colonization by the aflatoxin-producing fungi.
- E. To develop a rapid and reproducible procedure for the extraction of phenolic acids from peanut plant tissues.
- F. To identify and quantify free and bound phenolic acids in peanut seed coats and shells and relate their occurrence within resistant plant parts to *A. flavus* defense mechanisms.
- G. To investigate practical methods for the detection of mycotoxins in peanuts.
- H. To discover additional clay-related compounds which are capable of sorbing aflatoxin within peanut oil.

## ORGANIZATION

- |                           |  |
|---------------------------|--|
| A. U.S. Lead institution: | Texas A&M University   |
| Principal Investigator:   | Dr. Robert E. Pettit,<br>Department of Plant Pathology and Microbiology, TAES. |



- Co-Principal Investigators:** Dr. Timothy D. Phillips,  
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Dr. Olin D. Smith, Department of Soil  
and Crop Sciences, TAES.  
Mrs. Ruth Ann Taber  
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- Cooperators:** Dr. Juanito C. Reyes,  
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- Technician:** Mr. Ted A. Oplinger,  
Department of Plant Pathology and Microbiology, TAES
- Technical Assistants:** Mr. Aurelio Garcia and  
Miss Deborah Byars,  
Department of Plant Pathology and Microbiology, TAES.
- Budget Analysis:** Mrs. Tracy Robertson, Texas  
A&M Research Foundation, TAMU.
- International Coordinators:** Mr. James T. Goodwin, Texas  
A&M Research Foundation, TAES  
Ms. Violetta B. Cook, Texas A&M  
Research Foundation, TAES.
- International Representatives:** Dr. Neville P. Clarke  
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Dr. Dudley T. Smith,  
Associate Director, TAES.
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- Cooperator:** Mr. Jean Claude Mortreuil,  
CNRA, ISRA, IRHO, Bambey,  
Senegal B.P. 51
- Technician:** Mr. Emilien Sarr, Secteur  
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Senegal B.P. 199
- Directors:** Dr. Limamoulaye Cisse,  
Department des Production  
Vegetales, Bambey, Senegal  
B.P. 51  
Mr. Andre Rouziere, Secteur  
Centre-Sud, ISRA, Kaolack,  
Senegal B.P. 199

Director General: Dr. Moctar Touri, Institut  
Senegalais de Recherches  
Agricoles, Dakar, Senegal  
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C. Senegal counterpart Institution: 2. Institut de Technologie Alimentaire (ITA)

Principal Investigator: Dr. Amadou Kane, Mycotoxin  
Laboratoire, ITA, Dakar-Hann  
Senegal B.P. 2765.

Technicians: Mrs. Nassissatou Diop,  
Mr. Youssou Mane, and Mr.  
Souleymane Diack, ITA, Dakar  
Hann, Senegal B.P. 2765.

Director General: Dr. Mouhamadou Diop, ITA,  
Dakar-Hann, Senegal, B.P. 2765.

### Approach

A. Replicate field plot trials were conducted near Waller and Yoakum, Texas under rain-fed conditions within field soils known to contain *Aspergillus flavus* and *A. niger* propagules. Twenty peanut cultivars with previously observed degrees of tolerance to soilborne pathogens were planted in a randomized block design and replicated 4 times. Soil samples were taken monthly (June to November) to determine possible changes in the number of viable propagative units of *Aspergillus flavus* and *A. niger*. In addition, plant parts were sampled on nine occasions as follows: three times during the growing season, from the windrows immediately following digging, 1,2,3, and 4 weeks after digging, and following forced-air drying. Initially, aerial peanut pegs were sampled previous to their making contact with the soil surface. The second sampling consisted of pegs which had penetrated the soil. The third sampling consisted of immature pods approximately one-third of their potential size. The fourth sampling was from the inverted windrows immediately following digging. Additional plants were bagged at digging time from each plot and forced-air dried. Following drying, pod samples were obtained for plating on nutrient media. To insure that sufficient *Aspergillus flavus* was present, spores of the fungus were sprayed on the windrows shortly after digging. Pod samples were collected weekly, up to 4 weeks after digging. Plant parts were surface disinfested and plated on Griffin's nutrient medium.

B. Field plot trials were established near Niore, Senegal with a peanut variety SN 73-33, moderately tolerant to *Aspergillus flavus* invasion. The experiment consisted of 6 treatments where gypsum was applied on a 30 cm band over the peanut rows at 0, 15, 30, 45, and 60 days after planting at a rate of 600 kg/ha. A check treatment without gypsum was also included. Treatments were replicated 4 times in a randomized complete block design. All plots received mineral fertilizer (6-20-10) at a rate of 150 kg/ha. The incidence of *Aspergillus flavus* within the soil of each plot was monitored at 5 day intervals throughout the growing season. The soil pH was also determined periodically. The extent of pod and kernel invasion by *Aspergillus flavus* was assayed 15 days prior to harvest at 4, 15, 30 and 60 days after harvest. Aflatoxin analysis was performed using thin-layer chromatography.

C. In order to gain a more complete understanding of host-parasite interactions, the molecular weights of proteins within peanut kernels colonized by *Aspergillus flavus* or *Aspergillus parasiticus* were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Inoculated peanut kernels were removed from humidity chambers at 0, 3, 6, 9 and 12 days after inoculation for protein analysis. Testa-free cotyledons were ground in a buffer solution at 4C, filtered, and centrifuged at 20,000 g for 30 min. Anionic detergent—SDS (2.5% w/v) and 2-mercaptoethanol (5% v/v) were added and the samples heated at 100C for 5 min. Bromophenol blue was added as a front dye and samples subjected to discontinuous SDS-PAGE. The gel stacking zone was 4.5 T, 3% C, and the gradient zone was 8-25%. Proteins of known molecular weights were used as reference markers.

The gels were silver-stained and the molecular weights determined by construction of calibration curves.

D. The isoelectric points of proteins were determined by isoelectric focusing (IEF). Following preparation of samples (grinding cotyledons in buffer, filtering, and centrifugation) the proteins were separated on a gradient gel (pH 3-9) containing 5% T, and 3% C and compared with several known protein markers. Electrophoretic gels were also silver-stained.

E. The influence of drought stress on the induction of pathogenesis related proteins in cotyledonary tissue was determined on peanut cultivars J-11, SN 55-437, Toalson, TX 798736, and Starr. Plants were grown in microplots covered with a plastic greenhouse roof and subjected to two treatments: no drought stress and drought stress 100 days after planting. Harvested kernels were seeded with *A. flavus* or *A. parasiticus* spores and removed from humidity chambers at 6-hr intervals following inoculation. The proteins were separated from seeds by SDS, IEF, and 2-D electrophoresis. All cotyledonary proteins detected from the sound healthy kernels and those obtained from the inoculated kernels were mapped to determine the features of the pathogenesis related proteins. Proteins of fungal origin, detected in colonized cotyledonary tissue, were recorded and recovered separately.

F. An analysis of bound and free phenolic acids was conducted on the pods and testae of 14 peanut cultivars. Several extraction, filtration, and separating schemes were tested for their ability to accurately separate various phenolic acids using high pressure liquid chromatography (HPLC). Eighteen standard phenolic acids were also injected into the HPLC to assist in the determination of retention times and peak areas of the unknown compounds. Identification and quantification of the phenolic acids present within peanut shells of different ages and within peanut testae are in progress.

G. In order to determine the extent to which aflatoxin currently occurs in food products in Senegal, food samples were collected from the food markets in Dakar, Kaolack, and Diourbel, Senegal. A total of 478 food samples was collected and analyzed by the ITA staff for the presence of aflatoxin B1, using thin-layer chromatography.

H. A second experiment was conducted at ITA to determine the influence of cooking peanut butter (peanut paste) in water on reducing the aflatoxin level. Peanut paste from the Touba Toul market, containing 360 ppb aflatoxin, was mixed in water, with and without citric acid, and cooked for varying time periods. Cooking continued until the slurry resembled "mafe", a traditional food consumed in Senegal. Analysis for aflatoxin B1 was carried out using thin-layer chromatography with the BF method.

I. A third series of experiments at ITA involved the testing of clay materials for their ability to sorb aflatoxin in crude peanut oil. Crude peanut oil containing aflatoxin was obtained from Touba Toul (Thies district), Senegal, and subdivided into 100 ml samples. Two processed clays, bentonite and attapulgite, were added to the crude oil at rates of 5, 10, and 20% by weight. The clay was mixed with the oil on a shaker for 30 minutes, centrifuged, and the oil assayed for its aflatoxin content using thin-layer chromatography with a modified BF method.

J. A newly developed mycotoxin detection system with several desirable features (e.g. economical, stable, sensitive, and rapid), termed "Selectively Absorbed Mycotoxins" (SAM) was used to assay peanut samples received from the ICRISAT Sahelian research center near Niamey, Niger. Replicate samples of 10 peanut cultivars from different field-growing conditions were assayed using the SAM detection system, thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC). Comparisons were made concerning the accuracy of SAM as compared to TLC and HPLC.

#### ACCOMPLISHMENTS IN DETAIL

A. Field plot soil from experimental plots near Waller and Yoakum, Texas, contained *A. flavus* and *A. niger* levels sufficiently high to cause damage to peanut plants. The number of viable propagules ranged from 0 to 199 in soil samples from the Waller plots with a mean of 26 propagules per gram of soil. Mean levels increased during the growing season to a maximum of 6,849 propagules per gram of soil from Waller in October. *Aspergillus flavus* levels decreased rapidly in

November to 95-210 propagules per gram of soil. Yoakum soils contained higher mean levels which ranged from 300 per g of soil in June to 8,840 propagules per g of soil in October.

Recovery of *A. flavus* from peanut pegs, immature shells and mature shells, and kernels ranged from mean levels of 2.5% to 8.8% (Table 1). Aerial and soil-penetrated pegs contained similar *A. flavus* levels, with some increase following soil penetration. At harvest, kernel infestation levels ranged from 0.8% to 28.3%, with a mean of 8.8% for all peanut cultivars (Table 2). Following forced air drying, recovery rate of *A. flavus* ranged from 3.6% to 22.9%, with a mean level of 11.1%. Infestation levels under windrow conditions in the field, were lower. Kernels harvested the 1st, 2nd, 3rd, and 4th weeks after digging had mean infestation levels of 2.2, 2.5, 4.0, and 7.9% respectively.

Mean isolation frequencies of *A. flavus* from peanut plant parts collected from Waller and Yoakum field plots were lowest for the following cultivars: J-11, B815667 (a selection from a cross of Toalson/PI 365553/Tamnut-74), B804772 (a selection from a cross of Toalson/UF 73-4022), CV 171, and TX 798731 (a selection from a cross of Toalson/UF 73-4022) (Table 3). The highest mean levels of infestation were detected in plant parts from the 4 cultivars: TX 798736 (another selection from the cross Toalson/UF 73-4022), CV 402, Florunner and A72115.

B. Studies on the influence of gypsum application on the incidence of *A. flavus* in peanut soils near Nioro, Senegal revealed that gypsum did influence the activity of fungi in the pod zone of the peanut cultivar SN 73-33. Reduction of *A. flavus* soil populations was initially detected 15 days after gypsum applications. Population levels fluctuated throughout the growing season; however *A. flavus* levels at harvest time were lower in plots treated with gypsum 30, 45, and 60 days after planting. Fluctuations in soil pH averaged 0.5 pH units on these acidic soils (pH 4.3-4.5). *Aspergillus flavus* infestation levels within shells and kernels from all treatments 15 days prior to harvest and 4 days after harvest were relatively low. High levels of infestation were noted following 15 days of field curing in shells and kernels from the treatments: control, gypsum at sowing time, and gypsum applied 15 days after planting. Relatively low levels of *A. flavus* were detected in shells and kernels from the treatments on which gypsum was applied 30, 45, and 60 days after sowing. Aflatoxin levels were relatively low in kernels from all treatments, including the control treatment.

C. Thirty-four co-migrating groups of proteins were separated from sound peanut cotyledons of 13 cultivars using SDS-PAGE. Electrophoretic patterns of the proteins and differences between cultivars are illustrated in Table 4. Extremely low quantities of proteins with molecular weights (MW) between 102,300 and 218,700 daltons were identified by sensitive silver staining. Cotyledons of Florunner lacked a significant protein component (MW 37,100 daltons). SDS electrophoretic protein patterns revealed 5 major protein K at very high concentrations, 11 proteins at high concentrations, 9 proteins at moderate concentrations, and 7 proteins present at very low concentrations. The protein patterns of the cultivars Florunner, Starr, PI 341885, TX Ag 3, Toalson, SN 73-30, and PI 337409 differed.

D. Separation of the cotyledonary proteins using IEF revealed 40 protein bands within a pI range of 3.5-7.9. SDS-PAGE IEF, and 2-dimensional electrophoresis revealed that peanut kernels colonized by both aspergilli resulted in the decomposition of high molecular weight proteins (arachin, and conarachin fractions) into smaller molecular weight components, with a depletion of high molecular weight proteins. *Aspergillus flavus* decomposed the cotyledonary proteins more rapidly compared to *A. parasiticus*. (Fig 1.) Colonization by either fungus caused depletion of proteins from the acidic region of IEF gels, which increased with incubation time. There were differences within protein patterns of *Aspergillus* spp.-colonized cotyledons among peanut cultivars. Using SDS-PAGE, differences were recorded for the cultivars Florunner, Starr, TX Ag 3, and Toalson; using IEF, differences were recorded for the cultivars Florunner, Starr, PI 337409, and TX 798736.

**Table 1. Isolation Frequency of *Aspergillus flavus* Detected in Peanut Plant Parts from Plants Grown in a Field Plot near Yoakum, Texas**

Cultivar	Isolation frequency of <i>Aspergillus flavus</i> *				
	Aerial Pegs %	Soil Pegs %	Immature Pods %	Mature Shells %	Mature Kernels %
A72115	0.0 a**	6.0 b	11.0 a	4.2 ab	18.3 ab
PI365553/ Tamnut-74					
B855134	1.0 a	5.0 b	9.0 ab	0.0 b	11.7 ab
Toalson//PI 365553/ Tamnut-74					
B815667	4.0 a	3.0 b	8.0 abc	0.0 b	0.8 b
UF73-4022	2.0 a	4.0 b	3.0 abcd	5.0 ab	9.2 ab
CV402	5.0 a	5.0 b	4.0 bcd	0.0 b	17.5 ab
Toalson/UF 73-4022					
B804472	5.0 a	1.0 b	4.0 bcd	3.3 ab	21.2 ab
Toalson	1.0 a	21.0 a	4.0 bcd	0.0 b	4.2 b
Tamnut-74	2.0 1	5.0 b	4.0 bcd	0.0 b	9.2 ab
PI365553/ Tamnut-74					
B855010	2.0 a	3.0 b	4.0 bcd	5.0 ab	5.8 b
Toalson/UF 73-4022					
TX798736	5.0 a	6.0 b	3.0 bcd	0.0 b	28.3 a
J-11	0.0 a	1.0 b	3.0 bcd	1.7 b	1.7 b
Florunner	6.0 a	4.0 b	3.0 bcd	5.8 ab	1.7 b
PI337409	3.0 a	6.0 b	1.0 cd	10.0 a	5.8 b
Toalson/UF 73-4022					
TX798731	5.0 a	0.0 b	1.0 cd	2.5 ab	2.5 b
Starr	4.0 a	6.0 b	1.0 cd	0.0 b	9.2 ab
SN55-437	7.0 a	6.0 b	0.0 d	0.0 b	9.2 ab
Florunner/US 224					
B833809	1.0 a	1.0 b	0.0 d	7.5 ab	7.5 ab
Toalson/UF 73-4022					
B811956	2.0 a	1.0 b	0.0 d	0.0 b	2.5 b
CV171	3.0 a	4.0 b	0.0 d	0.0 b	1.7 b
PI365553/ Tamnut-74					
B855157	1.0 a	4.0 b	0.0 d	5.0 ab	8.3 ab
Mean	3.0	4.6	3.3	2.5	8.8

\*Isolation frequency reported as a mean of 4 replications where 25 plant parts were tested for each replication.

\*\*Means followed by the same letter are not significantly different at the 0.1 level (DNMR test).

**Table 2. Isolation Frequency of *Aspergillus flavus* Detected in Peanut Kernels from Plants Grown in a Field Plot near Yoakum, Texas**

Cultivar	Isolation frequency of <i>Aspergillus flavus</i> *					
	At Harvest %	Forced Air Dried %	Week 1 Windrow Drying %	Week 2 Windrow Drying %	Week 3 Windrow Drying %	Week 4 Windrow Drying %
Florunner	1.7 b**	3.6 e	1.4 a	0.7 b	13.6 ab	40.7 a
Florunner/US 224						
B833809	7.5 ab	16.4 abcd	3.6 a	1.4 b	2.1 bc	32.8 a
A72115	18.3 ab	9.3 cde	1.4 a	0.0 b	3.6 bc	20.0 b
Toalson/UF 73-4022						
B811956	2.5 b	9.3 cde	2.1 a	1.4 b	0.7 c	10.0 bc
UF73-4022	9.2 ab	11.4 bcde	1.4 a	4.3 ab	7.1 abc	10.0 bc
CV171	1.7 b	13.6 abcde	2.1 a	3.6 ab	15.7 a	8.6 bc
CV402	17.5 ab	17.9 abc	5.7 a	5.0 ab	5.0 bc	6.4 bc
Toalson/UF 73-4022						
TX798731	2.5 b	9.3 cde	0.0 a	2.1 b	3.6 bc	5.7 c
PI337409	5.8 b	7.1 cde	0.0 a	1.4 b	1.4 c	3.6 c
Toalson//PI 365553/ Tamnut-74						
B815667	0.8 b	5.7 de	0.0 a	1.4 b	0.0 c	2.9 c
PI365553/ Tamnut-74						
B855157	8.3 ab	22.9 a	5.0 a	7.9 a	2.1 bc	2.9 c
J-11	1.7 b	14.3 abcde	3.6 a	2.1 b	0.7 c	2.9 c
SN55-437	9.1 ab	9.3 cde	5.7 a	2.9 ab	8.6 abc	2.1 c
PI365553/ Tamnut-74						
B855134	11.7 ab	21.4 ab	2.9 a	2.9 ab	2.9 bc	2.1 c
Tamnut-74	9.1 ab	7.1 cde	0.7 a	2.1 b	4.3 bc	1.4 c
Starr	9.1 ab	10.7 bcde	0.0 a	0.7 b	2.1 bc	1.4 c
Toalson/UF 73-4022						
TX798736	28.3 a	11.4 bcde	4.3 a	3.6 ab	4.3 bc	1.4 c
PI365553/ Tamnut-74						
B855101	5.8 b	12.1 abcde	4.3 a	2.1 b	0.7 c	1.4 c
Toalson	4.2 b	5.7 de	0.0 a	2.9 ab	0.7 c	0.7 c
Toalson/UF 73-4022						
B804472	21.7 ab	4.3 e	0.0 a	0.7 b	1.4 c	0.0 c
Mean	8.8	11.1	2.2	2.5	4.0	7.9

\*Isolation frequency reported as a mean of 4 replications where 35 kernels were tested for each replication.

\*\*Means followed by the same letter are not significantly different at the 0.1 level (DNMR test).

**Table 3. Isolation Frequency of *Aspergillus flavus* and *Aspergillus parasiticus* from Peanut Plant Parts Collected in 1987**

Peanut Cultivar	Waller Samples %	Yoakum Samples %	Mean %
J-11	3.8 *	2.0	2.9
B815667	4.2	2.1	3.2
B804472	2.2	5.7	3.9
CV 171	3.8	4.2	4.0
TX 798731	4.5	3.6	4.1
Toalson	4.5	3.9	4.2
B855157	4.7	4.4	4.6
UF 73-4022	3.5	5.4	4.6
PI 337409	4.7	4.8	4.8
B855101	6.5	3.4	5.0
SN 55-437	5.3	5.1	5.2
B833809	5.4	6.5	6.0
TX 798736	3.8	8.3	6.1
CV 402	5.0	7.2	6.1
Florunner	5.4	8.0	6.7
A72115	10.1	8.2	9.2

\*Mean isolation frequency from 9 samplings of plant parts recovered from four replicated plantings of each peanut cultivar.

**Table 4. Electrophoretic Analysis of Peanut Cotyledon Proteins in 13 Cultivars**

Protein Molecular Weight	Cultivar* showing lack of certain protein band	Quantity** of protein
12,100	--	high
15,200	--	high
17,000	--	high
18,000	--	high
19,300	--	high
21,900	--	high
24,000	--	very high
26,900	--	moderate
28,800	--	moderate
30,000	--	moderate
30,900	--	high
33,100	--	high
34,700	--	high
37,100	Florunner	very high
40,700	--	very high
43,600	--	very high
51,500	--	moderate
56,200	--	moderate
60,200	--	moderate
65,300	--	very high
69,200	Starr, PI341885, TXAg 3, Toalson	low
71,600	Starr, PI341885, TXAg 3, Toalson	low
76,700	--	moderate
81,200	--	moderate
82,200	--	moderate
90,100	--	high
94,000	--	high
102,300	--	very low
109,600	--	very low
116,100	--	very low
120,200	--	very low
142,900	SN 73-30	very low
215,200	PI 337409	very low
218,700	PI 337409	very low

\*Cultivars investigated: Starr, PI 341885, PI 337409, Tamnut, Florunner, TXAg 3, Toalson, SN 55-437, TX 798636, SN 73-33, SN 73-30, Pronto, and PI 343419.

\*\*Quantity estimated according to intensity of protein bands according to scale: very high, high, moderate, low, very low.



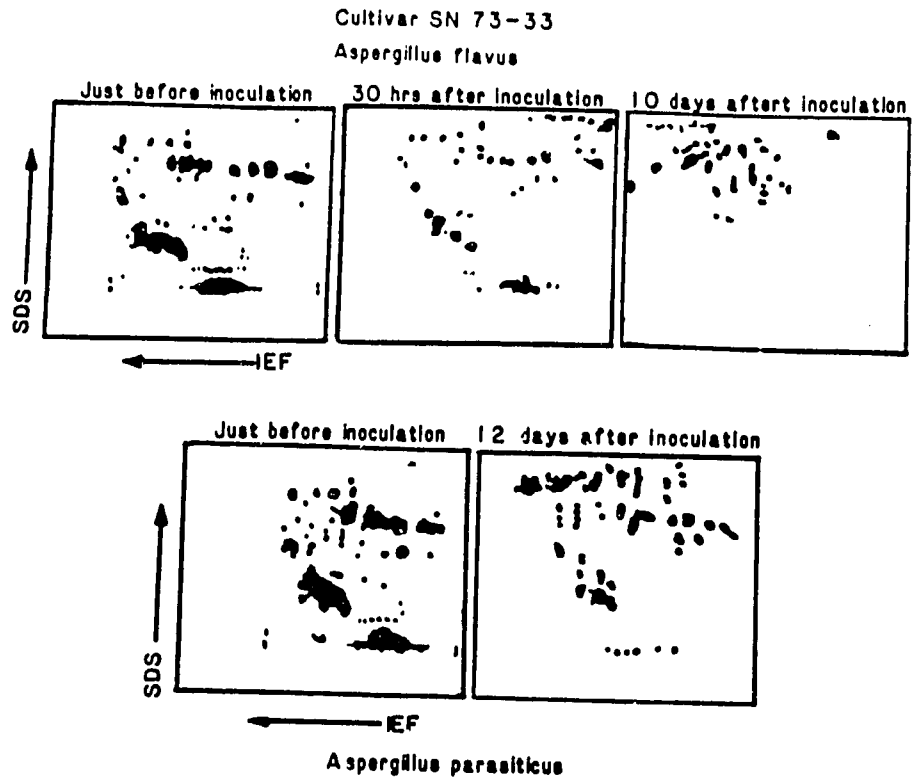


Figure 1. Protein patterns of cotyledons from kernels of SN 73-33 colonized by *A. flavus* and *A. parasiticus* for different periods of time (note more rapid decomposition of proteins by *A. flavus*)

E. Separation of proteins (using SDS-PAGE, IEF, and 2-dimensional electrophoresis) from sound mature kernels and *Aspergillus* spp. invaded kernels from plants grown under normal irrigation (NI) and drought stress (DS) revealed the presence of pathogenesis related (PR) proteins induced by the fungi. Synthesis of 4 PRs occurred within the time period of 24 hr after inoculation in all cultivars (J-11, SN 55-437, Toalson, TX 798736, and Starr) grown under NI conditions. Kernels from DS plants of each cultivar lacked PRs except from the cultivar TX 798736, which had 5 PRs (Fig.2). The concentration of these PRs increased during the next 12 hrs. Images of PRs became diffuse 37-42 hrs after inoculation. The presence or absence of these PRs, as molecular markers, is believed to have value in the characterization of different peanut cultivar, and may relate to the cultivars resistance or susceptibility to certain fungal pathogens.

F. An effective procedure for the extraction of phenolic acids from peanut shells and testae involved initial drying either by freeze-drying or oven drying at 40 C. Dried samples were ground in a Wiley Mill and 1.0g of tissue placed in polypropylene centrifuge tubes containing 30 ml of petroleum ether. These tubes were then shaken at low speed for 1 hr. They were then centrifuged for 5 min at 2,000 g and the petroleum ether discarded. To the residue 30 ml of 1% HCl in methanol (laboratory grade) was added, the samples shaken for 1 hr, and centrifuged for 10 min at 2,000 g. After removal from the centrifuge 20 ml of the acidic methanol was pipetted into 100 ml round bottom flasks in order to evaporate the solvent. After the acidic methanol was evaporated, 5ml of 4 N NaOH was added to the flasks to solubilize and remove the phenolic acids. The liquid suspension was decanted into a centrifuge tube and an additional 5 ml of 4 N NaOH was used to rinse the flask. The 10 ml of alkaline solution was shaken, placed in a desiccator, and a vacuum held for 15 min. The samples were held overnight and 10 ml of NaOH added to the residue the next day. The solution was neutralized with 6 N HCl and then 10 ml of ethyl acetate added. The solution was shaken for 3 min and centrifuged for 10 min at 2,500 g. The top phase (30 ml) was pipetted into round bottom flasks and evaporated to dryness. Then 5 ml of HPLC grade methanol was added to dissolve the phenolic acids. The solution was then filtered using a 0.45 membrane filter within a glass prefilter attached to a 5 ml syringe. The filtrate was placed in 4 ml vials, with a rubber stopper, for later HPLC analysis. Optimum HPLC separation was obtained by a multiple step gradient of the following solvent mixtures: (A) acetic acid-HPLC-grade methanol (2:98) and (B) butanol-HPLC-grade methanol (10:90). The HPLC detection was by ultraviolet absorption at 254 nm using a Beckman Model 153 analytical UV detector. Retention times and peak areas were obtained from a Hewlett Packard 3390 A reporting integrator. Examples of the type of chromatograms obtained for immature and mature shell tissue and mature testae tissue, are illustrated in Fig. 3. Similar chromatograms have been obtained for several different cultivars, and analyses of these data are in progress.

In order to determine the quantity of free phenols present within the acidic methanol fraction, 5 ml of the solvent was placed into test tubes and the Folin-Ciocalteu method used. The residue from the centrifugation of the acidic methanol fraction was saved for an analysis of bound phenols using an alkaline hydrolysis procedure.

G. The degree to which foodstuffs sold within Senegalese markets (e.g. Dakar, Kaolack, and Diourbel) are contaminated with aflatoxin B1 is summarized in Table 5. The highest mean concentrations of aflatoxin B1 were detected in peanut kernels (mean, 230 ppb), white corn (mean 124 ppb), yellow corn (mean, 65 ppb), peanut paste (butter) (mean, 62 ppb), and chocolate peanut butter (mean, 23 ppb). As noted in Table 5 a few samples of millet, grain sorghum, cowpea, and cassava contained aflatoxin (mean levels generally less than 20 ppb), with a maximum of 75 ppb in one millet sample. These data provide additional evidence that peanut and corn products pose the most serious health hazard in terms of their aflatoxin content. Other published evidence, from similar research throughout the world, indicates that these two commodities, peanuts and corn, consistently constitute one of the most serious problems in food and feed quality in terms of their aflatoxin contamination.

H. Attempts to reduce the aflatoxin levels present in peanut butter (by cooking) have been completed at IITA in Dakar. Peanut butter containing an initial level of 360 ppb aflatoxin B1 was cooked in water (with and without citric acid) for 1 and 2 hours. Following evaporation of the water,

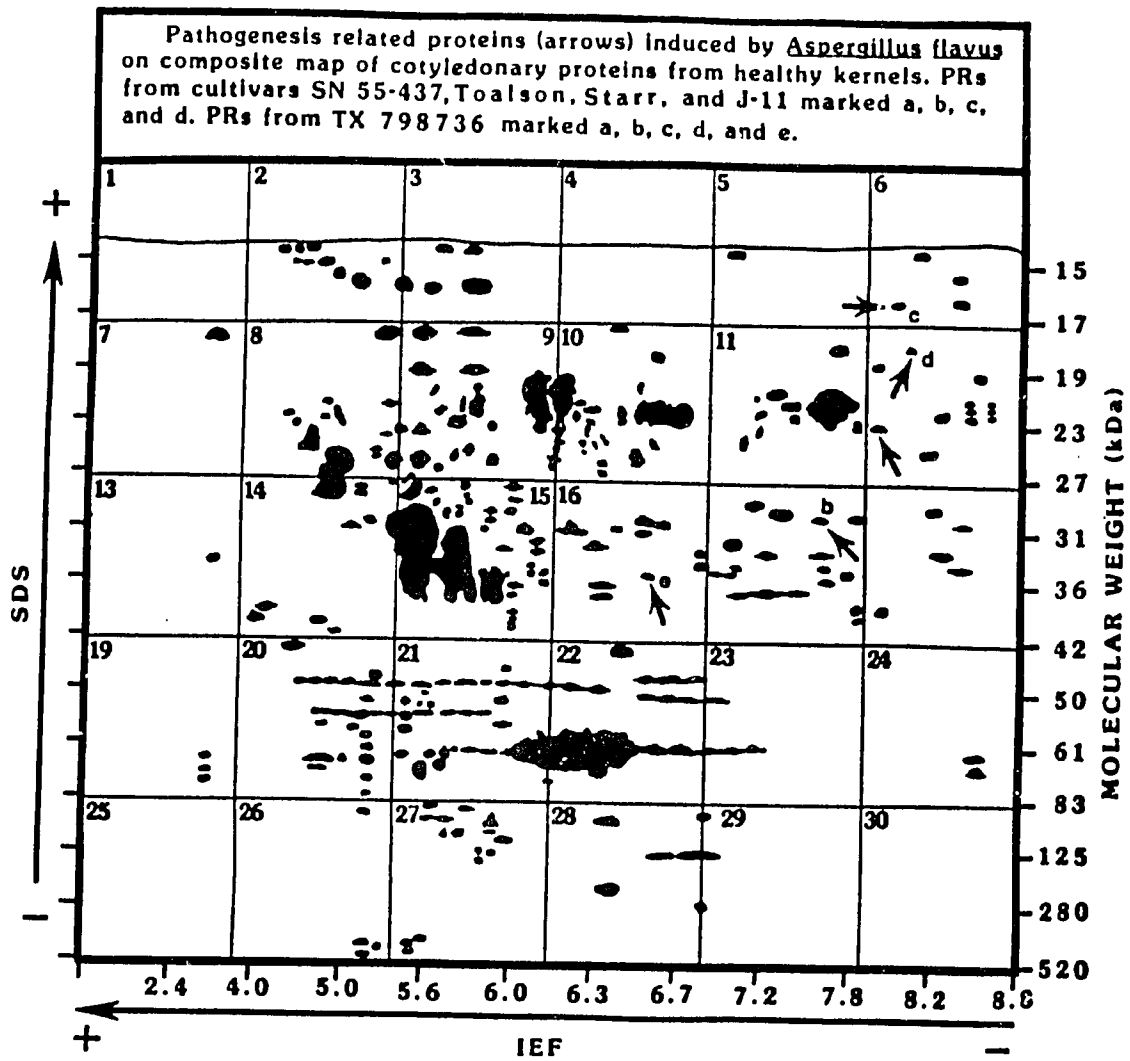
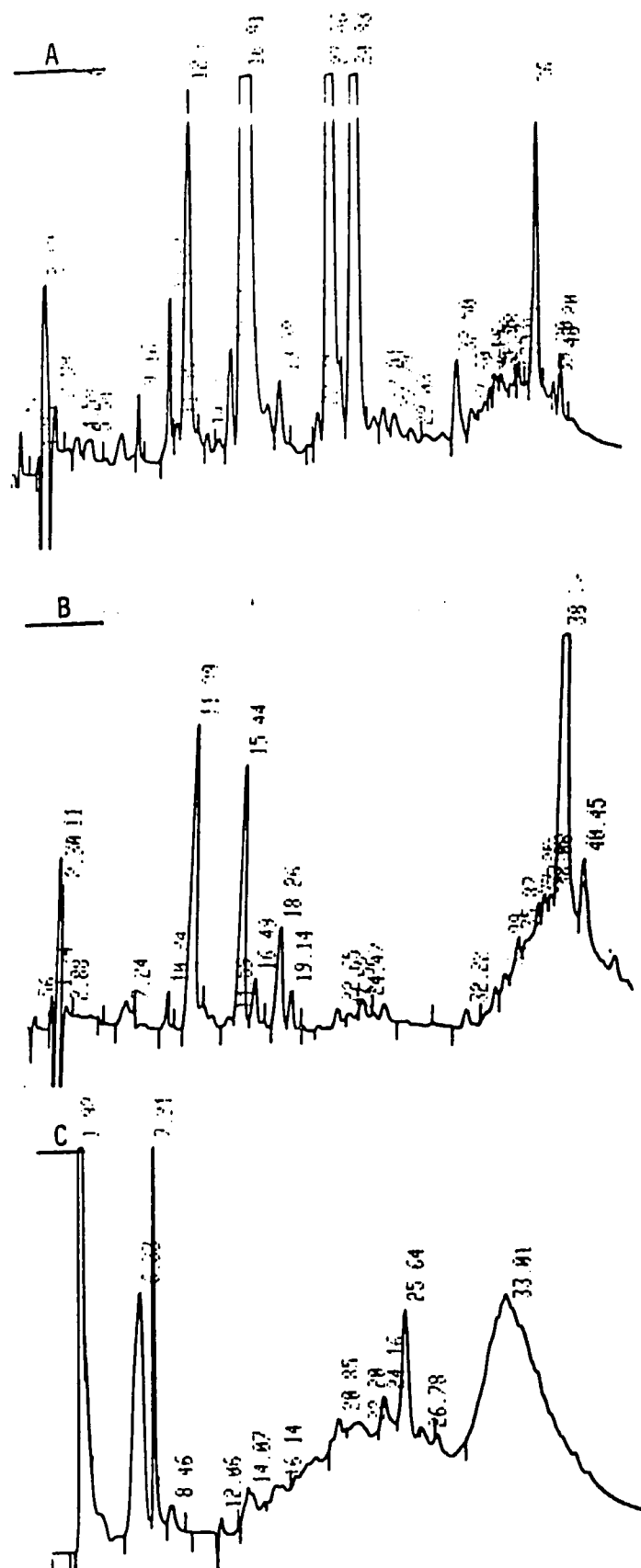


Figure 2. Pathogenesis-related proteins from kernels growing under normal irrigation and under drought stress



**Table 5. Occurrence of Aflatoxin B<sub>1</sub> Within Various Food Products from the Markets of Dakar, Kaolack, and Diourbel, Senegal West Africa**

Products	Number of samples	Number contaminated	Aflatoxin range - ppb	Aflatoxin mean - ppb
Mil Souna (entier) Millet intact	39	4 10%	3 - 75	2
Mil Souna (decortique) Millet hulled	15	0 0%	0	0
Couscous de mil Millet steamed	50	7 14%	1 - 39	2
Arachide (grines) Ground nut kernels	59	17 29%	2 - 4596	230
Pate d'arachide Peanut paste	22	12 55%	5 - 750	62
Chocoleca d'arachide Chocolate peanut paste	20	13 65%	1 - 125	23
Sorgho blanc White Grain Sorghum	36	14 39%	2 - 250	16
Sorgho rouge Red Grain Sorghum	25	0 0%	0	0
Mais blanc White corn	45	18 40%	1 - 1325	124
Mais jaune Yellow corn	42	25 60%	3 - 662	65
Riz Rice	24	1 4%	2	1
Niebe Cowpea	42	2 5%	16 - 17	1
Manioc Cassava	23	2 9%	3 - 8	1
Patatedouce Sweet Potato	12	0 0%	0	0

to create a peanut paste, the aflatoxin B<sub>1</sub> levels were determined by thin-layer chromatography. After cooking 200 g peanut butter in one liter of water for 1 h the residual aflatoxin level was 48 ppb, an 87% reduction in the aflatoxin B<sub>1</sub> present. Treatments which also contained citric acid were less effective in reducing the aflatoxin B<sub>1</sub> level; a residual level of 96 ppb was noted, only a 73% reduction. Increasing the cooking time to 2 hours failed to significantly reduce the aflatoxin level. These data indicate that cooking does not completely reduce the toxic potential of aflatoxin contaminated peanut butter; however, cooking procedures are capable of reducing the level of aflatoxin B<sub>1</sub> within a food.

I. Results from the crude peanut oil detoxification experiments in Senegal (at ITA) have continued and show considerable promise in terms of the ability of certain clays to sorb aflatoxin present within the oil. Processed bentonite and attapulgite clay materials when added to crude peanut oil were capable of removing over 90% of the aflatoxin B<sub>1</sub> present in the oil. Bentonite added at a rate of 10% by weight gave the greatest recovery rate of oil, 91%. In contrast, the addition of attapulgite at a rate of 10% resulted in a recovery of 76-88% of the original oil. Clay was removed in these experiments by centrifugation, a procedure which is not applicable to village conditions. The fine clay particles suspended within the oil are slow to settle.

J. The need for newly improved mycotoxin detection systems which do not require a high degree of technical expertise and laboratory sophistication (e.g. TLC, GLC, HPLC, GC/MS, etc.) has become more evident for the detection and molecular confirmation of mixtures of different mycotoxins in foodstuffs. The high costs and excessive time requirements associated with multi-mycotoxin analysis are prohibitive to good management. The development of rapid and inexpensive screening tests for mycotoxins including monocolumns and immunoassays have greatly facilitated diversion of mycotoxin contaminated grains, foods, and feeds through effective monitoring.

The most notable of these improved detection techniques employs enzyme-linked immunosorbent assays (ELISA). These tests do not require sophisticated laboratory equipment or extensive sample cleanup procedures and appear to be in excellent agreement with established methods for the analysis of single mycotoxins. However, much less attention has been directed to the development of practical techniques capable of screening food and feed for combinations of mycotoxins. When mycotoxins occur as mixtures they may potentially provoke complex interactions and toxic synergy. A simple multi-mycotoxin assay for the simultaneous detection of aflatoxins and zearalenone has been developed at Texas A&M in the Department of Veterinary Public Health and in collaboration with Rialdon Diagnostics of Bryan, Texas. This procedure is based on the concept of selective absorption of mycotoxins (SAM) onto specially prepared solids (patent pending). The SAM detector is constructed within a small tapered glass tube using a critical matrix of sorbent material that functions to detect interfering substances, selectively immobilize specific mycotoxins at targeted interfaces, and react at these interfaces to produce easily visualized fluorescent bands. The test for aflatoxins and zearalenone (SAM-AZ) includes four basic steps: extraction, cleanup, development, and detection, all of which can be accomplished in 10-15 minutes (Fig. 4). Fluorescence in the lower band of the SAM-AZ detector indicates a positive test for aflatoxins and fluorescence in the upper band indicates a positive test for zearalenone (Fig. 5). Further work is in progress to expand SAM's capabilities to detect tertiary and quaternary mixtures of mycotoxins using a combination of immunological and selective chemical absorption techniques in the cleanup phase to achieve enhanced levels of specificity. The SAM test is rapid, user-friendly, inexpensive, chemically stable, and does not require refrigeration, thus should be a highly desirable procedure for initial screening various grains, foods and feeds in developing countries.

In an experiment where the accuracy of SAM was compared with the results obtained using TLC and HPLC for aflatoxin detection, 19 peanut samples from Niger, West Africa were assayed. Following a modification of sample cleanup procedures, which included the use of an ether extraction step to remove an unidentified coeluting compound that interfered with aflatoxin, detection by HPLC was sensitive and accurate. The coeluting compound did not interfere with the SAM assay.

There was a 100% concordance between the HPLC and SAM assay procedures (Table 6). A sample which was positive for aflatoxins by HPLC was positive for aflatoxins by SAM and a sample which was negative by HPLC was also negative by SAM. It was concluded that SAM may provide a simple and practical means for screening a wide variety of peanut samples for aflatoxins. Unlike the ELISA technology, which requires refrigeration to maintain the integrity of critical enzymes in the test, SAM is chemically stable and has a very long shelf life.

#### TRAVEL

Robert E. Pettit and Ruth A. Taber. Traveled to Senegal to work with collaborators, Dr. Amadou Ba (ISRA) and Dr. Amadou Kane (ITA), during the period June 24-July 5, 1987.

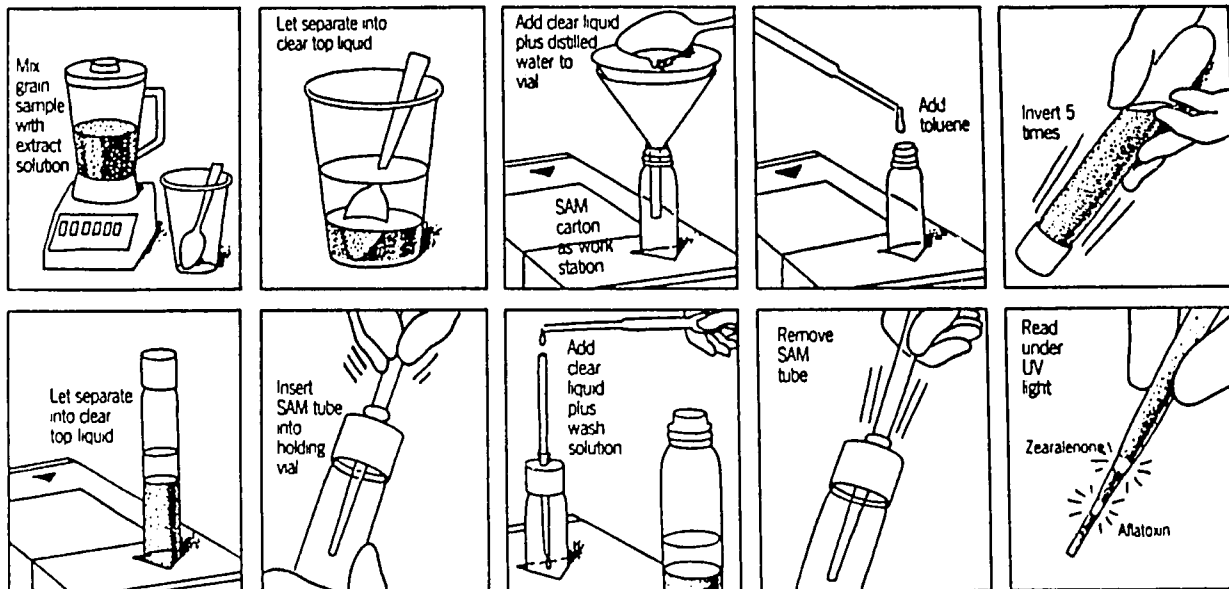
Robert E. Pettit and Bashir Sarr. Traveled to Orlando, FL to attend the annual meeting of the American Peanut Research and Education Society meeting, during the period July 13-17, 1987.

Robert E. Pettit and Amadou Ba. Traveled to Hyderabad, India to participate in the International Aflatoxin Workshop, during the period October 5-9, 1987.

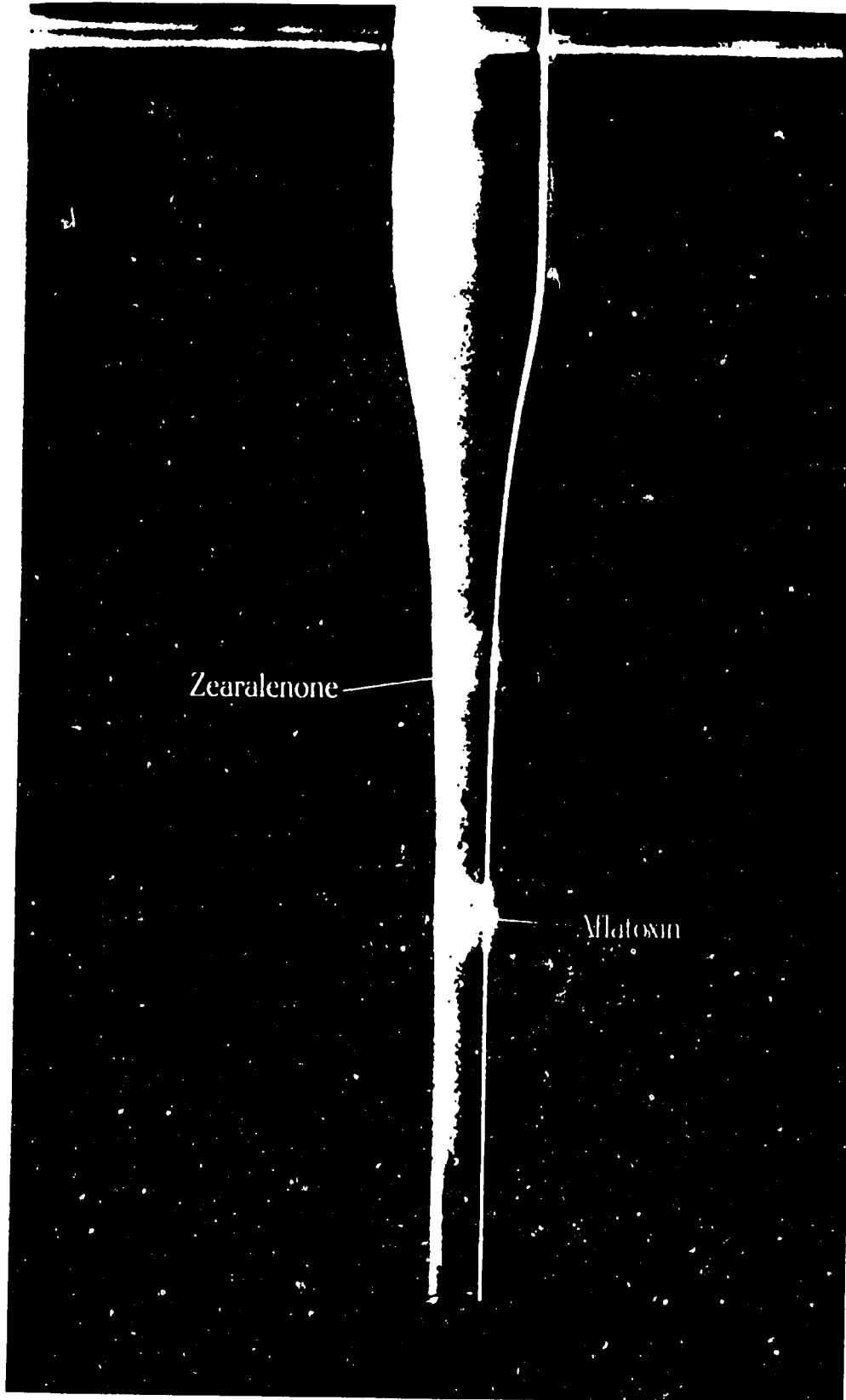
#### PRESENTATIONS

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Pettit, R. E., C. L. Martin, and O. D. Smith. 1986. Incidence of *Aspergillus flavus* and *A. niger* in peanut pegs, immature pods, and kernels. Proc. Amer. Peanut Res. and Ed. Soc. 18:64.

**Basic procedures for SAM-AZ Test. (See test kit for complete instructions)****Figure 4. General procedures for the SAM-AZ assay**





**Figure 5. SAM-AZ detector for the detection of aflatoxins and zearalenone from contaminated peanuts, corn, sorghum, wheat, soybeans, rice, oats, barley, and cottonseed**

**Table 6. Comparison of HPLC and SAM Analysis of Peanut Samples for Aflatoxins**

Sample Description <sup>a</sup> (Cultivars)	HPLC <sup>b</sup>				SAM <sup>c</sup>
	AFB <sub>1</sub>	AFB <sub>2</sub>	AFG <sub>1</sub>	AFG <sub>2</sub>	Aflatoxins
VAR 27	-	-	-	-	-
AH 7223	-	-	-	-	-
55 437	-	-	-	-	-
U1-2-1	+	+	-	-	+
J-11	-	-	-	-	-
VAR 27	-	-	-	-	-
28 206	-	-	-	-	-
TS 32-1	+	+	-	-	+
UF 71513	+	+	-	-	+
28 206	+	+	-	-	+
FAIZPUR	+	+	-	-	+
55 437	+	+	-	-	+
U4-47-7	+	+	-	-	+
J-11	+	+	-	-	+
U1-2-1	+	+	-	-	+
VAR 27	+	+	-	-	+
AH 7223	+	+	-	-	+
Unknown (Market Sample)	-	-	-	-	-
Unknown (Market Sample)	-	-	-	-	-

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#### PLANS FOR 1988-1989

A. Continue to study the ecology of *Aspergillus flavus* and *Aspergillus parasiticus* survival in soils as influenced by cultural practices.

B. Evaluate selected groundnut genotypes and cultivars in Senegal and Texas for their potential resistance to kernel infection by *Aspergillus flavus* and *Aspergillus parasiticus* and aflatoxin contamination under field conditions.

C. Examine peanut plant parts from field plots to determine the avenue of seed infection (preharvest) by *Aspergillus flavus* and *Aspergillus parasiticus*.

D. Continue a search for molecular components (e.g. tannic acids, pathogenesis related proteins, and isozymes) in resistant peanut genotypes and cultivars which could be of value in screening breeding lines for resistance to invasion by the aflatoxin producing fungi.

E. Continue the oil detoxification experiments at ITA using Novosil, attapulgite, bentonite, and other available clay-like materials for their ability to sorb aflatoxin in crude peanut oil.

F. Detoxification studies will also continue at Texas A&M using a new approach described in a paper published by Dr. Timothy D. Phillips (Phillips, T. D. et al. 1988. Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxin. *Poultry Sci.* 67:243-247). Emphasis will be placed on: (1) structural characterization of reaction products and (3) whole animal toxicity.

G. Detoxification studies will continue at ITA on the development of procedures to reduce the levels of aflatoxin in peanut paste (butter) and other peanut products consumed in Senegal using heat and different additives.

H. Efforts will continue on the testing of mycotoxin detection procedures which are quick, accurate, economical, and capable of detecting more than one mycotoxin within peanut kernels or processed food, or feed products.

## IPM Strategies for Peanut Insects in SAT Africa

University of Georgia-University of Ouagadougou, Burkina Faso

R. E. Lynch, Principal Investigator, UGA

### INTRODUCTION

Food production in Semi-Arid Tropical (SAT) Africa is characterized by large fluctuations, both between years and between areas within a year. These fluctuations in crop production result in inadequate food supplies and subsistence farming, even though the region contains vast areas of arable land suitable for crop production. Thus, stability in crop production has been designated as one of the priorities for SAT Africa.

Inadequate and/or irregular rainfall is one of the major factors contributing to the fluctuations in crop production in SAT Africa. Equally important are the losses due to arthropod pests and plant diseases, lack of improved crop varieties, agronomic practices, and cropping systems.

Recent reviews by Amin and Mohammad (1980), Wightman (1985), and Lynch et al. (1986) have discussed the major groundnut pests in the SAT. In SAT Africa, the groundnut aphid (*Aphis craccivora* Koch), jassids (*Empoasca dolichi* Paoli, and *E. facialis* Jacobi), an armyworm [*Spodoptera littoralis* (Boisduval)], the groundnut hopper (*Hilda patruelis* Stal), a termite (*Microtermes thoracalis* Sjostedt), millipedes (*Peridontopyge* spp.), the groundnut bruchid [*Caryedon serratus* (Ol.)], and the "Wang" [*Aphanus* (= *Elasmolomus*) *sordidus* (F.)] are considered major pests of groundnuts (Amin and Mohammad 1980). Lynch et al. (1986) identified termites, millipedes, thrips, and jassids as potential economic pests in Burkina Faso. Of these, termites are the most serious pest since they not only reduce groundnut yield (Feakin 1973; Johnson et al. 1981; Sudhakar and Veeresh 1985), but also enhance *Aspergillus flavus* (Link) infection (McDonald and Harkness 1963, 1964; McDonald et al. 1964; McDonald 1969; Pollet and Decleret 1987).

### MAJOR ACCOMPLISHMENTS

#### Research Results

Four major research objectives were addressed in the 1987 groundnut research in Burkina Faso. These included: 1) Determination of yield losses due to arthropods via control of certain insects or groups of insects with insecticides; 2) Determination of the effect of groundnut harvest date on termite and millipede damage and subsequent aflatoxin contamination of kernels; 3) Evaluation of groundnut lines from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) with reported resistance to termites; 4) Evaluation of neem leaves, seed, and cake for control of groundnut arthropods.

In Georgia, three major research objectives were addressed: 1) Evaluation of insect-resistant peanut lines from ICRISAT for insect resistance in Georgia; 2) Evaluation of advanced peanut breeding lines for insect resistance and susceptibility; and 3) Evaluation of alternate food sources for pinto beans in the meridic diet of the fall armyworm.

In Burkina Faso, Temik, Lorsban, and Temik plus Lorsban significantly reduced the percentage defoliation of plants due to insects, percentage of thrips damage, and the percentage of termite-penetrated pods. Yield was increased only by the season-long control of insects where both Temik and Lorsban were applied. Both millipede and termite damage to groundnut plants increased with time after planting. Termite damage to pods was significantly greater for pods harvested at 110 days than for pods harvested earlier. Delayed harvest to 125 days increased termite damage even more and substantially increased the aflatoxin content of kernels. Groundnut cultivars NC Ac 343, NC Ac 2240, and RMP 40 had the least termite damage, even with delayed harvest, while maintaining yields

comparable with local varieties. Ground neem leaves and seed applied in a granular formulation significantly reduced thrips damage to groundnuts but had little influence on jassids, lepidopterous defoliators, termite damage to pods, or yield.

In Georgia, several peanut lines identified at ICRISAT as resistant to insect damage sustained lower leafhopper-damage and defoliation ratings than the standard U.S. variety, 'Florunner'. Four of the ICRISAT lines produced yields comparable to Florunner. However, Florunner had a significantly higher percentage of total sound mature kernels (TSMK) than that for the other lines evaluated. Evaluation of 16 U.S. advanced breeding and commercial lines for insect damage produced significant differences in thrips, leafhopper, and velvetbean caterpillar damage. Peanut, peanut meal, soybean meal, bermudagrass pellets, and alfalfa pellets were evaluated as substitutes for pinto bean in the meridic diet used for rearing the fall armyworm. Bermudagrass pellets, alfalfa pellets, and peanut meal were suitable substitutes for pinto bean.

### Training

Idrissa Ousmane Dicko, Ph.D. candidate in the Department of Entomology at the University of Georgia, passed his oral and written preliminary examinations and has completed all course-work requirements. All data for a groundnut life table in relation to insect abundance and damage have been collected and are being analyzed. He is expected to defend his dissertation in April or May 1989.

A budget increase proposal was submitted to the Peanut CRSP Board of Directors to fund Mr. Solibo Some of the University of Ouagadougou in his Ph.D. program in the Department of Entomology at the University of Georgia. Pending approval by the Board of Directors, Some should begin his classwork beginning the spring quarter of 1989.

### EXPECTED IMPACT OF THE PROJECT

The identification of economic insect pests of peanut in Burkina Faso and determination of yield losses attributable to these pests are the first steps in the development of an integrated pest management program. Research on yield losses for these insects will define economic injury levels in relation to the socio-economics for Burkina Faso and can be used to develop economic thresholds. The relationship between insect damage to peanut pods and aflatoxin contamination is also vitally important to the health of both human and livestock consumers of peanut and peanut products, but does not readily lend itself to economic analysis. However, these data will aid in the development of IPM strategies that will reduce losses to arthropods or reduce aflatoxin contamination and, thus, aid in a more stable agricultural production for SAT Africa. Furthermore, the research conducted in Burkina Faso can be extrapolated and/or used as a model for insect damage and losses in other SAT countries.

### GOAL

Identify the major arthropod pests of peanut in Burkina Faso, develop economic thresholds for these pests, develop IPM strategies and control measures to reduce losses to these pests, and determine the relationship between arthropod damage to peanut pods and the incidence of aflatoxin contamination.

### OBJECTIVES

- A. Identify the major economic pests of peanut.
- B. Determine the relationship between level and type of arthropod damage with aflatoxin contamination in both preharvest and postharvest peanut.
- C. Develop economic injury levels for the major arthropod pests by quantifying pest density with peanut yield.

- D. Develop reliable sampling procedures to estimate population densities of the major pests.
- E. Determine arthropod abundance as related to peanut growing season and developmental phenology.
- F. Provide training opportunities for Burkina Faso students.
- G. Develop bait attractants or other control strategies for major insect pests.
- H. Evaluate promising breeding lines developed by the Breeding CRSP for resistance-susceptibility to major arthropod pests.

## ORGANIZATION

### University of Georgia

Dr. Robert E. Lynch, Principal Investigator, Insect Biology and Population Management Research Laboratory, Tifton, Georgia.

### Institute Superior Polytechnique (ISP)

Dr. Albert Patoin Ouedraogo, Collaborating Principal Investigator, University of Ouagadougou, Burkina Faso.

Mr. Solibo Some, Assistant Collaborator, University of Ouagadougou, Burkina Faso.

### Approach

During the initial stages of the program, research was focused on the following objectives:

- A. Survey the arthropod problems of groundnut in Burkina Faso to relate arthropod densities with peanut developmental phenology.
- B. Evaluate local peanut cultivars for arthropod damage using two different cultural practices common to Burkina Faso.

These objectives have been fulfilled.

In the second stage, research is directed toward the following objectives:

- A. Determination of the impact of arthropods on groundnut yield through utilization of aldicarb for control of thrips and jassids during the early part of the growing season, and chlorpyrifos for control of termites and millipedes during the latter part of the growing season.
- B. Evaluate the effects of harvest date and insect damage on yield and aflatoxin contamination in groundnuts.
- C. Evaluate groundnut lines identified by ICRISAT as resistant to termites in India for resistance to termites and aflatoxin in Burkina Faso.
- D. Evaluate neem products, i.e., leaves, kernels, cake, and oil, for insect control on groundnuts in the field.
- E. Evaluate neem products for insect control in stored groundnuts.

## ACCOMPLISHMENTS IN DETAIL

### Burkina Faso

#### Yield Losses and Damage Due to Arthropods

Table 1 presents data on the chemical control of groundnut insects and the effects on groundnut yield. Temik, a systemic insecticide, was applied at planting and at planting + at pegging for thrips and jassid (= leafhoppers) control. Thrips damage for both of these treatments was reduced significantly in comparison to thrips damage in the untreated control, but no significant differences were noted among treatments in jassid damage. Likewise, neither of the Temik treatments improved groundnut yield compared to yield for the untreated control.

Granular Lorsban was applied at pegging and at pod fill to control soil insects such as millipedes and termites. These insects tend to occur during the latter one-half of the growing season and primarily damage groundnut pods. Lorsban alone significantly increased the percentage of undamaged pods and significantly reduced the percentage of termite-penetrated pods when compared to these pod damage categories for the untreated control. However, Lorsban alone did not result in a significant yield increase over the yield of the untreated control. Only a combination of Temik for thrips and jassid control plus Lorsban for soil arthropod control significantly increased groundnut yield over the yield for the untreated control. In this test, the yield loss due to groundnut insects was 30 percent (yield of plots treated with Temik + Lorsban - yield of untreated plots/yield of untreated plots).

#### Groundnut Harvest Date

Table 2 presents data on the effects of groundnut harvest date on millipede damage, termite damage, and yield. There were no significant differences among treatments in the number of plants at thinning. However, there were significantly fewer plants at harvest for plots harvested at 125 days than there were for plots harvested at 70 or 90 days or for the chemical control. Millipede damage to plants was minimal with only a maximum of 4.9 percent damage at 125 days. Termite damage to plants was more substantial; plants harvested at 110 days had significantly more termite damage than plants harvested at 70 or 90 days or the chemical control, and plants harvested at 125 days had significantly more termite-damaged plants than all other treatments.

Termite damage to groundnut pods increased significantly between 90 and 110 days, but increased even more dramatically between 110 and 125 days. There were no significant differences in the percentages of undamaged pods, externally scarified pods, or penetrated pods for plants harvested at 70 or 90 days or for the control. However, plants harvested at 110 days had a significantly lower percentage of undamaged pods and a significantly higher percentage of penetrated pods than plants harvested at 70 or 90 days or for the treated control plants. Likewise, delayed harvest to 125 days significantly decreased the percentage of undamaged pods and significantly increased the percentage of scarified and penetrated pods over those for all other treatments.

Groundnut yield was greatest for the control plants treated for insect control, significantly greater than plants harvested at 70, 90, or 125 days. Inappropriate harvest, whether too early (70 and 90 days) or too late (125 days), substantially reduced yield.

Table 3 presents data on the effects of harvest date on incidence of *Aspergillus niger*, *A. flavus*, and aflatoxin. *A. niger* incidence was significantly greater on pods harvested at 70, 90, and 125 days than on pods from the chemical control. Kernel contamination with *A. niger* was significantly greater for the delayed, 125-day harvest than for kernels from all other treatments. All treatments had a significantly higher incidence of *A. flavus* on pods than did pods from the treated control. Pods from the delayed also had a significantly higher incidence of *A. flavus* than pods from the 70- and 110-day harvest. Kernel contamination with *A. flavus* was significantly greater for the delayed harvest of 125 days than kernel contamination for all other treatments. This relationship appears directly related to the level of termite damage to groundnut pods prior to harvest. Furthermore, aflatoxin B<sub>1</sub>, aflatoxin B<sub>2</sub>, and total aflatoxin were significantly higher in kernels from the delayed harvest than aflatoxin in kernels from any of the other treatments. Plants harvested at 70 and 90 days also had kernels contaminated with aflatoxin, which probably reflects poor drying conditions after harvest. Aflatoxin



concentration in kernels from these plots was approximately 15 times lower than aflatoxin concentrations in kernels from plants harvested at 125 days. Thus, delayed harvest greatly increases the level of termite damage to groundnut pods which enhances invasion by *A. flavus* and formation of aflatoxin.

#### Evaluation of Termite-Resistant Groundnut Lines

Results for the evaluation of groundnut lines from ICRISAT with pod resistance to termites are presented in Table 4. Two harvest dates, a normal harvest and a delayed harvest, were employed to enhance termite damage to groundnut pods. Plant damage was recorded only for the first harvest as breakage of dead plants and dry soil during the second harvest made evaluation of plant damage impossible. Both millipede and termite damage to plants were minimal for the first harvest date. Millipede damage was significantly greater for QH 243, one of the local varieties, than for all except one of the other cultivars evaluated. NC Ac 2142 also sustained significantly more termite damage than several of the other cultivars. Least plant damage by millipedes was recorded for NC Ac 2242 and NC Ac 2243. Plant damage by termites was most severe for M13, a susceptible cultivar. No termite damage to plants was noted on Robut 33-1, NC Ac 343, NC Ac 2240, NC Ac 2242, NC Ac 2243, and NC Ac 10033.

Significant cultivar x harvest date interactions were noted for all pod damage classes, indicating that damage to pods by termites differed among cultivars for plants harvested on each of the two dates. Therefore, analyses were performed and results are presented by individual harvest date (Table 4). For the first harvest, RMP 40 sustained the least pod scarification and pod penetration, significantly less than 8 of the 14 cultivars evaluated. NC Ac 343, NC Ac 2242, NC Ac 2243, NC Ac 2240, and M13 also had significantly more undamaged pods than NC Ac 17888, NC Ac 10033, NC Ac 2142, or QH 243. QH 243 had the highest percentage of externally scarified pods, and QH 243 and NC Ac 2142 had the highest percentage of termite-penetrated pods for the first harvest.

For the second harvest, NC Ac 2243 had the highest percentage of undamaged pods, lowest percentage of scarified pods, and lowest percentage of penetrated pods (Table 4), possibly as a result of low plant populations for this cultivar. NC Ac 2240, RMP 40, and NC Ac 343 also had significantly more undamaged pods than NC Ac 10033, M13, NC Ac 17888, and QH 243. Robut 33-1 (susceptible) and Bonga (local cultivar) had significantly more scarified pods than all other cultivars evaluated. Robut 33-1 also had a significantly higher percentage of penetrated pods than all other cultivars. With the exception of NC Ac 2243, pods of NC Ac 2240, NC Ac 2142, RMP 40, and NC Ac 343 were among the least damaged.

Groundnut yield for the first harvest was greatest for NC Ac 343, significantly greater than for NC Ac 2240, NC Ac 2142, Robut 33-1, QH 243, RMP 40, NC Ac 2242, and NC Ac 2243. For the second harvest, yield for NC Ac 10033 was significantly greater than for NC Ac 2242 or NC Ac 2243.

Overall, RMP 40, NC Ac 2240, NC Ac 2243, NC Ac 343, and the local cultivar, Bonga, showed the least termite damage to plants and pods while, with the exception of NC Ac 2243, maintaining acceptable yields. Amin et al. (1985) rated NC Ac 2240 and NC Ac 2243 resistant and RMP 40 and NC Ac 2243 moderately resistant to termite pod scarification. Results of this study may be somewhat biased for NC Ac 2243 since its low plant population may have contributed to its low termite damage and reduced yields. NC Ac 343, in particular, shows promise with reduced termite damage for both harvests, the highest yield for the first harvest, and yield within 6.3% of the highest yield for the second harvest. NC Ac 343 is resistant to pod damage by the southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) and moderately resistant to foliage-feeding insects (Campbell et al. 1971), thrips (*Frankliniella fusca* Hinds), and leafhoppers (*Empoasca fabae* Harris) (Campbell and Wynne 1980).

Termite damage to groundnut and associated aflatoxin contamination of kernels are among the most serious problems in many parts of West Africa. Both termite damage and aflatoxin are exacerbated by irregular or inadequate rainfall, especially during the latter part of the growing season (Johnson and Gumel 1981; Johnson et al. 1981). In Burkina Faso, both the beginning and the end of the rainy season pose problems for crop production. If rain is delayed at the beginning of the rainy

season, groundnuts may not have time to mature. Late planting results in more immature pods during the latter part of the growing season. Kernels in these immature pods are more susceptible to *A. flavus* invasion and aflatoxin contamination during inadequate rain that often occurs in the late growing season (Sanders et al. 1981; Hill et al. 1983; Blankenship et al. 1984). Similarly, erratic rainfall during the latter part of the growing season increases the probability of termite damage to groundnut plants and pods, *A. flavus* invasion of pods, and aflatoxin contamination of kernels (McDonald 1969; Feakin 1973; Johnson and Gumel 1981; Johnson et al. 1981; Sanders et al. 1981). Continued research on the relationships among harvest date, termite damage, *A. flavus* invasion of pods, aflatoxin formation in kernels, and evaluation for termite resistance in groundnut cultivars offers potential for greatly reducing the insect and aflatoxin problems in West Africa.

#### Evaluation of Neem for Arthropod Control

Table 5 presents data on the effects of neem on groundnut insects and insect damage in the field. Neem leaves or neem seed were ground into a fine powder, mixed with corncob granules, and applied over the row with a granular applicator for evaluation against an untreated control and a treated control where Temik and Lorsban were applied for insect control. Significant differences among treatments were noted in thrips/10 terminals, thrips damage, jassids/10 sweeps, jassid damage, percent defoliation, millipede damage to plants, termite damage to pods, and yield. Major differences, for the most part, were between the untreated control and treated control; Temik and Lorsban significantly reduced the thrips damage, jassid damage, and defoliation. In general, application of ground neem seed resulted in slightly lower insect populations, although not always significantly lower, than insect populations in the untreated control.

#### Georgia

##### Evaluation of Groundnut Lines for Insect Resistance

In Georgia, eleven ICRISAT lines were compared to the commercial line Florunner for insect damage, yield and grade (Table 6). Robut 33-1 was the most susceptible to thrips damage with a significantly higher damage rating than 8 of the lines evaluated. Robut 33-1 and ICGPRS-103 also had the highest leafhopper (jassid) damage ratings, significantly higher than ICGPRS-1, ICGPRS-35, ICGPRS-93, or JL 24. Defoliation by velvetbean caterpillar, *Anticarsia gemmatilis*, was significantly greater for JL 24 than for all other lines evaluated with the exception of Florunner. ICGPRS-96, ICGPRS-103, and Robut 33-1 sustained the least amount of defoliation, significantly less than JL 24, Florunner, ICGPRS-1, or ICGPRS-35. Significant differences were also noted among lines in the grade characteristics, i.e., total sound mature kernels (TSMK), extra large kernels (ELK), and weight of 100 sound mature kernels, but none of the varieties compared favorably with Florunner in the percentage of sound mature kernels. However, this difference in percentage of TSMK may reflect inappropriate harvest dates for optimum maturity for the ICRISAT lines since several of these lines produced yields comparable with Florunner.

Table 7 presents data on the evaluation of advanced peanut breeding lines in comparison with standard commercial varieties (Florunner, 'Florigiant', and 'New Mexico Valencia C') and known insect-resistant varieties (NC 6 and Tifton 8) for insect damage, yield, and grade. Thrips damage was greatest on UF 85179, significantly greater than damage on 11 of the lines evaluated. Three lines, UF 85410, NC 18417, and New Mexico Valencia C, had lower thrips damage ratings comparable to the resistant checks. Leafhopper damage was most severe on UF 82107, significantly greater than all but two of the varieties evaluated. The resistant varieties, NC 6 and Tifton 8, had the lowest leafhopper damage ratings. However, leafhopper ratings for only three lines, UF 82107, UF 851033, and UF 85179, were significantly greater than the resistant checks. Velvetbean caterpillar defoliation was significantly greater for VP 8140 than defoliation for 8 of the lines evaluated. Tifton 8, one of the insect resistant varieties, sustained the least defoliation; eight of the advanced breeding lines had defoliation ratings that did not differ significantly from the ratings for Tifton 8. Yield was greatest for UF 82107, significantly greater than for all other lines evaluated, with the exception of UF 79308-1. In general, the percentage of TSMK was greatest for the runner lines and probably reflect more optimum harvest for these lines.

### Laboratory Rearing of Insects

Tables 8, 9, 10, and 11 present results of various meridic diets on the biology of the fall armyworm. Bermudagrass pellets, alfalfa pellets, and peanut meal were suitable substitutes for pinto beans in the meridic diet for rearing the fall armyworm. Diets containing these ingredients promoted rapid larval weight gain and development, pupae of normal weight, excellent survival to adults, and excellent fecundity. Insects reared on raw peanuts had reduced survival, and insects reared on soybean meal had reduced fecundity.

### Plans for 1988

#### (A) Research

Five major research thrusts are planned for Burkina Faso. These include 1) determine yield losses due to arthropod damage to groundnuts by application of insecticides to control these arthropods; 2) evaluate the effect of groundnut harvest date on termite damage to groundnut and subsequent aflatoxin contamination; 3) evaluate groundnut lines from ICRISAT with termite resistance for damage and aflatoxin formation in Burkina Faso; 4) evaluate neem leaves and seed for insect control in the field; and 5) evaluate neem leaves, seed, and oil for insect control in stored peanuts.

### TRAINING AND TRAVEL

Dr. Albert Patoin Ouedraogo, University of Ouagadougou, Burkina Faso, visited the U.S. from July 14-28, 1987. He attended the APRES Annual Meeting in Orlando, FL, and attended the Peanut CRSP meeting that followed. He then returned to Tifton, GA, to work with Dr. Robert E. Lynch and review the peanut entomology research program.

Dr. Robert E. Lynch attended the APRES Annual Meeting in Orlando, presented a paper on the effect of peanut stripe virus on yield and grade of Florunner peanuts, and attended the Peanut CRSP meeting. In November, he traveled to Burkina Faso to review the 1987 research results and plan the 1988 research program.

### PRESENTATIONS

Effect of peanut stripe virus on yield of Florunner peanut. 1985. Natl. Conf. Entomol. Soc. Amer., Reno, NV. Peanut soil insects. 1987. Peanut Pest Management School, Tifton, GA.

Peanut stripe virus research: 1985-1986. 1987. Univ. of Georgia Interdisciplinary Committee on Peanut Stripe Virus, Byron, GA.

Effect of peanut stripe virus on yield and grade of Florunner peanut. 1987. APRES Annual Meeting, Orlando, FL.

Insect management and control. 1988. Peanut Short Course, Tifton, GA.

Effect of peanut stripe virus on Florunner peanut. 1988. 52nd Ann. Meeting of the Georgia Entomological Society, Statesboro, GA.

West Africa. 1988. Presentation to 3rd grade students at G. O. Bailey Elementary School, Tifton, GA.

Effect of groundnut harvest date and termite-resistant varieties on termite damage in Burkina Faso, West Africa. 1988. 20th Ann. Meeting of the American Peanut Research and Education Society, Tulsa, OK.

Effect of harvest date and termite-resistant varieties on termite and millipede damage to groundnut in Burkina Faso. 1988. Invited presentation at the Groundnut Production in West Africa Symposium, ICRISAT Sahelian Center, Niamey, Niger.

New ways to get bad bugs. 1988. Second Ann. Georgia Peanut Tour, Plains, GA.

Predicting infestations of the lesser cornstalk borer. 1988. Georgia Agricultural Commodity Commission for Peanuts Meeting, Tifton, GA.

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Table 1. Chemical control of groundnut arthropods and effects on groundnut yield in Burkina Faso, 1987.<sup>1/</sup>

Treatment <sup>2/</sup>	Rate (kg/ha)	Number plants/m	Number pods/m	Defoliation (%)	Thrips damage (%)	Jassid damage (%)	Pod damage (%)			Yield (kg/ha)
							Undamaged	Externally scarified	Penetrated	
1. Untreated control	0	8.0ab	211.7c	56.7a	58.3a	13.3a	94.0bc	2.9b	3.0a	1151.3b
2. Temik @ planting	5.6	8.8ab	275.3bc	28.3c	30.0b	8.3a	92.5c	4.8a	2.5a	1250.4ab
3. Temik @ planting @ pegging	5.6 5.6	9.3a	330.3a	40.0b	30.0b	13.3a	95.7ab	3.7ab	0.6b	1178.7b
4. Lorsban @ pegging @ pod fill	7.5 7.5	8.0ab	299.5ab	20.0c	31.7b	10.0a	97.2a	2.3b	0.5b	1253.6ab
5. Temik @ planting @ pegging	5.6 5.6	7.7b	247.8bc	18.3c	23.3b	8.3a	96.8a	2.8b	0.4b	1498.3a
Lorsban @ pegging @ pod fill	7.5 7.5									

<sup>1/</sup> Means within a column followed by the same letter are not significantly different ( $p < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> The at planting application was applied in-furrow. The pegging application was applied 40 days after planting, and the pod fill application was applied 40 days after the pegging application.

Table 2. Effect of harvest date on millipede and termite damage to groundnut in Burkina Faso, 1987.<sup>1/</sup>

Harvest date (DAP) <sup>2/</sup>	No. plants at		% plants with <sup>4/</sup>		Termite-damaged pods (%) <sup>4/</sup>			Yield	
	thinning	harvest	Millipede damage	Termite damage	Undamaged	Scarified	Penetrated	(g/plot)	Loss (%) <sup>4/</sup>
70	309.3a	295.2a	5.5a	0.0c	98.8a	0.9bc	0.4c	4033.3d	-38.1
90	314.0a	291.0a	3.1bc	0.5c	97.8a	1.5bc	0.8c	5533.3bc	-15.1
110	310.5a	285.2ab	3.1bc	3.8b	92.5b	5.5b	2.0b	5916.7ab	- 9.2
125	318.8a	266.5b	4.9ab	17.6a	40.3c	55.2a	4.6a	4883.3c	-25.1
Control <sup>3/</sup>	297.2a	290.2a	1.5c	0.0c	99.2a	0.7c	0.2c	6516.7a	-

<sup>1/</sup> Means within a column followed by the same letter are not significantly different ( $p < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> DAP = days after planting.

<sup>3/</sup> The control was treated with 0.84 AI/ha aldicarb at planting and with 1.20 AI/ha chlorpyrifos at pegging and 50 days later, and was harvested at 100 DAP.

<sup>4/</sup> Percentage data converted to  $\arcsin \sqrt{\%}$  for analysis.

Table 3. Effect of groundnut harvest date on the presence of Aspergillus niger, A. flavus, and aflatoxin in Burkina Faso, 1987.<sup>1/</sup>

Harvest date (DAP) <sup>2/</sup>	% pods with <sup>4/</sup>		% kernels with <sup>4/</sup>		Aflatoxin (ppb) <sup>5/</sup>				Total
	<u>A. niger</u>	<u>A. flavus</u>	<u>A. niger</u>	<u>A. flavus</u>	B1	B2	G1	G2	
70	20.6a	9.4b	3.9b	0.4b	39.2b	7.3b	--	--	46.5b
90	25.7a	18.6ab	6.8b	2.7b	96.3b	14.0b	--	--	110.3b
110	15.8ab	9.2b	10.8b	5.0b	0.0b	0.0b	--	--	0.0b
125	40.8a	25.9a	34.7a	26.9a	1419.7a	185.8a	--	--	1605.5a
Control <sup>3/</sup>	2.8b	1.7c	0.6b	1.0b	0.0b	0.0b	--	--	0.0b

<sup>1/</sup> Means within a column followed by the same letter are not significantly different ( $p < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> DAP = days after planting.

<sup>3/</sup> The control was treated with 0.84 AI/ha aldicarb at planting and with 1.20 AI/ha chlorpyrifos at pegging and 50 days later, and was harvested at 100 DAP.

<sup>4/</sup> Percentage data converted to  $\arcsin \sqrt{\%}$  for analysis.

<sup>5/</sup> Data converted to log 10 for analysis.



Table 4. Effects of variety and harvest date on termite and millipede damage to groundnut in Burkina Faso, 1987.

Groundnut variety	% Millipede		% Termite		Undamaged pods		Scarified pods		Penetrated pods		Yield (g/plot) <sup>2/</sup>	
	damaged plants		damaged plants									
	Oct. 10 <sup>1/</sup>	Nov. 5 <sup>1/</sup>	Oct. 10	Nov. 5	Oct. 10	Nov. 5	Oct. 10	Nov. 5	Oct. 10	Nov. 5	Oct. 10	Nov. 5
M13	0.94 bcd	--	1.16 a	--	94.9 abcd	70.0 ef	1.0 bcde	3.0 bcd	4.1 def	27.0 b	891.7 ab	515.0 ab
TMV2	1.26 bcd	--	0.64 ab	--	91.6 def	77.5 cde	1.3 abcd	2.9 bcd	7.2 bcd	19.6 cdef	616.7 abcd	411.7 abc
Robut 33-1	1.82 bc	--	0.00 b	--	88.8 f	51.0 g	0.7 bcde	9.3 a	10.1 b	39.7 a	471.7 cde	348.3 abc
NC Ac 343	1.14 bcd	--	0.00 b	--	95.9 abc	82.8 bc	0.2 de	1.7 de	3.9 def	15.6 efg	950.0 a	550.0 ab
NC Ac 2142	2.25 ab	--	0.59 ab	--	82.4 g	76.3 cde	1.8 ab	1.3 de	15.8 a	22.3 bcde	495.0 bcde	553.3 ab
NC Ac 2230	1.25 bcd	--	0.75 ab	--	92.3 cdef	77.5 cde	1.3 abc	5.2 b	6.4 cde	17.3 defg	740.0 abcd	541.7 ab
NC Ac 2240	0.71 cd	--	0.00 b	--	95.4 abc	87.3 ab	0.4 cde	0.9 de	4.2 def	11.8 gh	508.3 bcde	360.0 abc
NC Ac 2242	0.25 d	--	0.00 b	--	96.4 ab	79.0 cd	0.0 e	2.7 bcde	3.6 def	18.3 cdefg	333.3 de	245.0 bc
NC Ac 2243*	0.00 d*	--	0.00 b*	--	95.5 abc*	92.3 a*	1.3 abc*	0.0 e*	3.2 ef*	7.8 h*	162.5 e*	97.5 c*
NC Ac 10033	1.38 bcd	--	0.00 b	--	89.6 ef	70.2 ef	0.8 bcde	4.6 bc	10.0 b	25.3 bc	650.0 abcd	586.7 a
NC Ac 17888	1.34 bcd	--	0.25 ab	--	91.0 ef	67.5 f	0.9 bcde	3.4 bcd	8.1 bc	29.1 b	878.3 abc	531.7 ab
RMP 40	1.08 bcd	--	0.63 ab	--	97.7 z	82.9 bc	0.6 cde	2.5 bcde	1.8 f	14.7 fg	447.5 de	446.7 ab
Bonga (local)	0.95 bcd	--	0.24 ab	--	93.2 bcde	74.8 cdef	0.3 cde	8.0 a	6.5 cde	17.3 defg	616.7 abcd	401.7 abc
QH 243 (local)	3.42 a	--	0.87 ab	--	80.7 g	73.4 def	2.2 a	2.0 cde	18.8 a	24.6 bcd	465.0 cde	310.0 abc

<sup>1/</sup> Harvest date: Oct. 10 = ca. 100 days after planting; Nov. 5 = ca. 125 days after planting.

<sup>2/</sup> Yield for 2 rows, each 10 meters in length, with 1 seed planted every 10 cm and 30 cm between rows.

\* Inadequate seed of NC Ac 2243 resulted in only Rep I and one-half of Rep II being planted, which may bias the results for this variety.

Table 5. Evaluation of neem for control of groundnut insects in Burkina Faso, 1987.<sup>1/</sup>

Treatment	Rate (g/plot)	Number plants/m	Thrips/10 terminals	Thrips damage (%)	Jassids/10 sweeps	Jassid damage (%)	Lepid./m	Defol. (%)	Millipede damaged plants (%)	Termite damaged plants (%)	Termite-damaged pods (%)			Yield (kg/ha)
											Undam.	Externally scarified	Penetrated	
Untreated control	0	9.4a	81.6a	66.0a	18.2a	48.0a	0.8a	12.0a	0.9a	0.4a	97.0a	1.5b	1.5ab	1128.8a
Neem leaves @ pegging	21.6	9.6a	53.2bc	46.0b	13.8ab	38.0ab	0.8a	12.0a	0.1b	0.1a	97.0a	1.6b	1.4ab	1276.8a
Neem leaves @ pegging	108.0	8.6a	36.8c	46.0b	15.2ab	36.0ab	0.4a	15.0a	0.1b	0.0a	96.4ab	1.6b	2.1ab	1008.5a
Neem leaves @ post 70	21.6	8.8a	61.0ab	50.0b	10.2ab	46.0a	0.6a	12.0a	0.1b	0.0a	97.0a	1.5b	1.6ab	980.7b
Neem leaves @ post 70	108.0	9.0a	42.0bc	46.0b	12.0ab	40.0ab	0.2a	11.0a	0.2b	0.0a	94.9b	3.3a	2.0ab	1110.3a
Neem seed @ pegging	21.6	8.8a	32.4c	42.0b	11.6ab	30.0b	0.2a	10.0a	0.1b	0.0a	97.4a	1.0b	1.6ab	1069.8a
Neem seed @ pegging	108.0	8.8a	28.6cd	46.0b	13.2ab	38.0ab	0.2a	12.0a	0.3b	0.2a	96.5ab	1.9b	1.7ab	1045.5a
Neem seed @ post 70	21.6	9.2a	46.2bc	44.0b	9.8ab	36.0ab	0.2a	10.0a	0.2b	0.0a	96.4ab	1.9b	1.8ab	1040.9a
Neem seed @ post 70	108.0	9.2a	32.8c	38.0b	11.6ab	36.0ab	0.2a	11.0a	0.1b	0.0a	95.8ab	1.7b	2.5a	1050.1a
Temik + Lorsban	<sup>2/</sup>	9.8a	8.2d	9.0c	5.6b	9.0c	1.0a	5.0b	0.0b	0.1a	96.9a	2.1b	1.2b	1288.3a

<sup>1/</sup> Means followed by the same letter are not significantly different ( $P < 0.05$ ) using Duncan's new multiple range test.

<sup>2/</sup> Temik applied at 5.6 kg/ha at planting and at pegging, and Lorsban applied at 7.5 kg/ha at pegging and 40 days later for insect control.

Table 6. Evaluation of peanut lines from ICRISAT for insect resistance in Georgia, 1987.<sup>1/</sup>

Peanut line	Thrips <sup>2/</sup> damage rating	Leafhopper <sup>2/</sup> damage rating	Velvetbean <sup>2/</sup> caterpillar defoliation	TSMK <sup>3/</sup> (%)	ELK <sup>3/</sup> (%)	Weight/ 100 SMK <sup>3/</sup> (g)	Yield (kg/ha)
ICGPRS-1	4.7b	4.3bc	3.3bc	71.5c	20.5d	63.2ef	3316.1ab
ICGPRS-35	4.7b	4.0c	3.3bc	71.9c	34.5a	70.1ab	3256.2abc
ICGPRS-43	5.3ab	4.7abc	3.0cd	72.0c	26.1bc	61.1f	2724.1bcd
ICGPRS-61	4.7b	4.7abc	3.0cd	71.3c	26.2bc	64.3de	2615.0bcd
ICGPRS-92	4.3b	4.7abc	3.0cd	74.4b	35.3a	70.8a	3946.0a
ICGPRS-93	5.0ab	4.3bc	3.0cd	62.9d	28.8b	66.6cd	2395.1cd
ICGPRS-96	4.3b	5.3ab	2.7d	71.3c	24.8c	67.0bcd	3848.1a
ICGPRS-103	4.7b	5.7a	2.7d	71.8c	36.6a	68.0abc	2626.6bcd
ICGPRS-215	5.0ab	4.7abc	3.0cd	62.0d	28.5b	69.5abc	2077.6d
Robut 33-1	6.0a	5.7a	2.7d	76.0b	19.6de	55.4g	3853.6a
JL 24	4.7b	4.3bc	4.0a	72.0c	7.3f	51.7h	2435.3cd
Florunner	4.3b	5.0abc	3.7ab	78.0a	16.8e	55.2g	3749.2a

<sup>1/</sup> Means within a column followed by the same letter do not differ significantly ( $p < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> Insect damage rated on a 1-9 scale, where 1 = no damage and 9 = severe damage.

<sup>3/</sup> TSMK = Total sound mature kernels; ELK = Extra large kernels; SMK = Sound mature kernels.

Table 7. Insect damage, yield, and quality characteristics of runner (RU), virginia (VA), and valencia (VAL) types of peanuts when no insecticides are used, Tifton, Georgia, 1987. <sup>1/</sup>

Variety	Market type	Yield (lb/acre)	Thrips <sup>2/</sup> damage	Leafhopper <sup>2/</sup> damage	Velvetbean caterpillar defoliation <sup>2/</sup>	Fancy pods (%)	ELK <sup>3/</sup> (%)	TSMK <sup>3/</sup> (%)	Meats (%)	DK <sup>3/</sup> (%)	OK <sup>3/</sup> (%)	Seed wt. (g/100)
Florunner	RU	3122cde	4.8ab	4.0cd	3.0cd	0.8h	12.3d	73.8bc	78.0a	0.2cde	4.0ab	54.9fg
VP 8140	RU	3132bcd	4.3bc	3.7d	3.8a	1.8h	0.0e	73.2cd	76.2c	0.2de	2.8cde	54.9fg
GA T-2524	RU	2918de	4.3bc	4.2bcd	3.0cd	0.0h	0.0e	73.0cd	77.5b	0.1e	4.4a	42.0h
UF 79308-1	RU	3413ab	4.3bc	4.0cd	3.7ab	1.1h	14.8d	75.6ab	78.5ab	0.2cde	2.6cdef	58.2ef
UF 87315	RU	2980ie	4.3bc	4.0cd	3.3abcd	0.7h	13.3d	77.5a	79.1a	0.3cde	2.5defg	54.6fg
TP 107-11	RU	2874e	4.5abc	4.0cd	3.3abcd	0.6h	11.9d	74.8bc	78.4ab	0.4cde	3.2bcd	55.2fg
Florigiant	VA	2888de	4.5abc	4.2bcd	3.2bcd	43.1e	26.9c	68.1f	70.6fg	0.5bcde	2.0efgh	79.6b
NC 18417	VA	3060cde	4.0cd	4.2bcd	3.3abcd	46.8de	13.2d	67.4f	70.1g	0.3cde	2.4defg	72.8c
GA T-2449	VA	2896de	4.2c	4.0cd	3.2bcd	52.2cd	25.7c	64.9g	68.2h	0.9bcd	2.5defg	64.4d
UF 82107	VA	3476a	4.3bc	5.0a	3.5abc	54.4bc	32.3b	68.7f	71.2fg	0.9lc	1.6fgh	81.1b
UF 85410	VA	3298abc	4.0cd	4.2bcd	3.2bcd	58.6b	42.2a	71.3d	73.8d	0.9bc	1.7fgh	86.8a
NC 6	VA	2924de	3.5d	3.7d	3.0cd	67.6a	42.1a	71.1de	72.8de	0.3cde	1.4h	88.0a
Tifton 8	VA	2577f	4.0cd	3.7d	2.8d	30.3f	34.2b	69.0ef	71.2fg	0.6bcde	1.5gh	77.8b
New Mexico Valencia C	VAL	2006g	4.0cd	4.0cd	3.7ab	4.3h	0.0e	68.7f	72.7de	0.4cde	3.5bc	44.1h
UF 851033	VAL	1977g	4.8ab	4.8ab	3.7ab	11.0g	0.0e	62.6h	71.6ef	4.5a	4.6a	51.4g
UF 85179	VAL	2195g	5.0a	4.7abc	2.8d	10.5g	4.1e	68.4f	71.9ef	1.2b	2.3defgh	60.9de

<sup>1/</sup>Means within a column followed by the same letter do not differ significantly at the 95 percent level of probability.

<sup>2/</sup>Insect damage rated on a scale from 1-9, where 1 = no damage and 9 = severe damage.

<sup>3/</sup>ELK = Extra large kernels; TSMK = Total sound mature kernels; DK = Damaged kernels; OK = Other kernels.

Table 8. Effects of substituting diet ingredients for pinto bean in the fall armyworm meridic diet on fall armyworm larval development.

Diet treatment <sup>2/</sup>	10-d larval weight (mg)	Survival at 10 d (%) <sup>3/</sup>	Developmental parameters <sup>1/</sup>			
			Days to pupation	Pupal weight (mg)		Survival to pupation (%) <sup>3/</sup>
				Male	Female	
Pinto bean	187.3b	100.0a	14.7c	246.5a	257.9a	92.5a
Peanut	90.5c	82.0b	19.9a	202.1b	92.2b	11.5c
50% P:50% PB	73.6d	98.0a	18.1b	223.0ab	232.0a	60.0b
Bermudagrass pellets	236.9a	98.0a	14.2d	256.9a	264.7a	97.0a
50% BP:50% PB	233.0a	100.0a	14.2d	252.8a	262.3a	96.5a

<sup>1/</sup> Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> 50% PB:50% P = 50% pinto bean:50% peanut; 50% PB:50% BP = 50% pinto bean:50% bermudagrass pellets.

<sup>3/</sup> Percentage data transformed to  $\arcsin \sqrt{\%}$  for analysis.

Table 9. Effects of substituting diet ingredients for pinto bean in the fall armyworm meridic diet on fall armyworm adult biology and fecundity.<sup>1/</sup>

Diet treatment <sup>2/</sup>	Days to adults	Survival to adults (%) <sup>3/</sup>	Sex ratio male:female	No. days ovipositing <sup>4/</sup>	Egg masses/female	Eggs/female	Eggs/female/day
Pinto bean	23.2c	85.5b	1.18:1.00a	5.7a	7.2a	1003.6a	176.5ab
Peanut <sup>5/</sup>	29.0a	1.0d	1.00:1.00a	-	-	-	-
50% PB:50% P	27.3b	29.0c	1.07:1.00a	5.0a	7.9a	704.3b	137.4b
Bermudagrass pellets	22.7c	92.5a	1.00:1.00a	5.6a	6.0a	1093.5a	199.8a
50% PB:50% BP	22.6c	91.5ab	1.00:1.08a	5.7a	6.3a	1070.6a	188.5a

<sup>1/</sup> Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> 50% P:50% PB = 50% peanut:50% pinto bean; 50% BP:50% PB = 50% bermudagrass pellets:50% pinto bean.

<sup>3/</sup> Percentage data transformed to  $\arcsin \sqrt{\%}$  for analysis.

<sup>4/</sup> Oviposition data based on 6 pairs of adults/replication and 10 replications.

<sup>5/</sup> Insufficient survival on peanut precluded oviposition data.

Table 10. Effects of substituting diet ingredients for pinto bean in the fall armyworm meridic diet on fall armyworm larval development.

Diet treatment	10-d larval weight (mg)	Survival <sub>2</sub> at 10 d (%) <sup>2/</sup>	Developmental parameters <sup>1/</sup>			
			Days to pupation	Pupal weight (mg)		Survival to pupation (%) <sup>2/</sup>
				Male	Female	
Pinto bean	248.8b	92.5ab	14.4b	264.2a	281.7ab	90.0a
Soybean mean	295.2a	88.0b	14.0c	240.0c	257.3c	87.4a
Peanut meal	171.8d	97.0a	14.8a	248.9b	262.9c	93.4a
Bermudagrass pellets	203.0c	89.7b	14.5b	264.3a	286.8a	90.0a
Alfalfa pellets	210.2c	89.6b	14.8a	263.2a	276.8b	90.5a

<sup>1/</sup> Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> Percentage data transformed to  $\arcsin \sqrt{\%}$  for analysis.

Table 11. Effects of substituting diet ingredients for pinto bean in the fall armyworm meridic diet on fall armyworm adult biology and fecundity.<sup>1/</sup>

Diet treatment	Days to adults	Survival to adults (%) <sup>2/</sup>	Sex ratio male:female	No. days ovipositing <sup>3/</sup>	Egg masses/female	Eggs/female	Eggs/female/day
Pinto bean	22.7c	90.0a	1.00:1.08ab	6.2a	8.0a	844.3a	133.1a
Soybean meal	22.1d	87.4a	1.00:1.25ab	6.4a	6.3a	526.4b	81.2b
Peanut meal	23.1b	93.4a	1.00:1.44a	6.2a	8.0a	924.4a	149.6a
Bermudagrass pellets	23.1b	90.0a	1.25:1.00bc	6.1a	7.0a	1001.0a	163.8a
Alfalfa pellets	23.4a	90.5a	1.53:1.00c	6.6a	7.8a	917.6a	141.9a

<sup>1/</sup> Means within a column followed by the same letter are not significantly different ( $P < 0.05$ ) using Duncan's multiple range test.

<sup>2/</sup> Percentage data transformed to  $\arcsin \sqrt{\%}$  for analysis.

<sup>3/</sup> Oviposition data based on 6 pairs of adults/replication and 10 replications.



## Management of Arthropods on Peanuts in Southeast Asia

W.V. Campbell, Principal Investigator, NCSU

### INTRODUCTION

Insects, spider mites and millipedes comprise an arthropod complex that breeds continuously on peanut in Southeast Asia. This pest complex attacks the foliage, stems, and pods and transmits important viruses to peanuts. These arthropods are a major constraint on peanut production and yield in Thailand and the Philippines. A better knowledge of the major pests and their damage potential is needed to develop an environmentally safe and economically sound pest management program. This research is intended to provide information to strengthen existing pest management programs in Thailand and the Philippines.

### MAJOR ACCOMPLISHMENTS

#### Research Results

**Thailand.** An international collection of 250 peanut genotypes were evaluated in uniform nursery tests in 1986 in North Carolina, Philippines and Thailand. Genotypes were reduced to 150 in 1987 and retested in Thailand and North Carolina. Peanuts were identified with resistance to thrips, leafhopper, leaf miner, pod damage caused by the subterranean ant and yellow spot virus resistance transmitted by the thrips *Scirtothrips dorsalis*. Some genotypes possessed multiple pest resistance and 77 genotypes gave higher yields than the standard local cultivar Tainan 9. Several high yielding, insect resistant genotypes were incorporated into a pilot pest management experiment to be conducted in several locations in 1988 to determine the advantage of pest resistant germplasm in an IPM program.

Prediction equations were developed to assess the effect of insect damage on unfilled pods and seed yield. Losses from unfilled pods and seed yield losses were related to foliage damage from leafminer, leafhopper and thrips at the R<sub>4</sub> and R<sub>5</sub> plant growth stages. This information is important to the IPM program to identify pests responsible for yield loss and the phenological stages of the plant most susceptible to foliage damage from specific pests.

**Philippines.** Entomology research at UPLB supported by Peanut CRSP is intended to strengthen the IPM programs now being used. Research includes biological control with insects and insect pathogens, the damage potential of important pests and evaluation of pest management programs. Three species of the egg parasite *Trichogramma* reduced defoliation by lepidopterous pests and increased yields, although yields were not significantly different. Significant yield losses from the cutworm *Spodoptera litura* were related to number of larvae per plant and the plant phenological stage at the time of defoliation. Two strains of *Bacillus thuringiensis* (B.t.) were highly effective against 3rd instar *S. litura* larvae under field conditions; however the half life is less than three days. *Bacillus thuringiensis* is not compatible with all pesticides and reduced rates of pesticides were required to prevent loss of virulence of B. t.

**North Carolina.** An international collection of 150 insect resistant peanut genotypes were retested for multiple pest resistance in North Carolina and Thailand. Multiple pest resistance was confirmed among many selected genotypes. It was common among peanuts with insect resistance to be resistant to several species of the same insect genera. However, insect resistance in some peanuts was species specific. This international collection of peanuts that was selected for pest resistance in North Carolina, Thailand and the Philippines was further reduced from 150 entries to 24 entries to establish a Uniform Insect Resistance Nursery with cooperation from Georgia (USDA), Georgia (University), Florida, Alabama and Virginia. This Uniform Insect Resistance Nursery will provide area wide exposure of selected genotypes to a complex of insects. Pheromones are generally useful to monitor insects to determine population peaks and seasonal history data. In North Carolina the southern corn

rootworm (SCR) pheromone 10-methyl-2-tridecanone is employed also to provide information for pest management decisions. Data indicate a good relationship between SCR trap catches and SCR pod damage. If trap counts average more than 100 SCR adults/week during a four-week period in June then it would be profitable to apply a pesticide for SCR control. Data are now being collected for further confirmation of this preliminary threshold. Selected insect resistant germplasm from the international collection with multiple insect resistance was evaluated for cross resistance to post harvest moth complex. Appropriate field selected susceptible checks and local cultivars were included in the test. Germplasm susceptible to preharvest insect pests was susceptible to the Indian meal moth and almond moth complex in the laboratory. More than 50% of the entries had fewer larvae and less damage than the susceptible checks. These data suggest secondary compounds or volatiles that attract or deter preharvest pests may also attract or deter post harvest pests. Chemical analyses are needed for identification of preharvest and post harvest volatiles.

#### EXPECTED IMPACT OF PROJECT

**Philippines and Thailand.** Develop a pest management program from information generated from Peanut CRSP and incorporate into the IPM program insect resistant peanut germplasm identified in Uniform Insect Nursery Tests that were established in Thailand and the Philippines. The development by the breeder of agronomically acceptable, pest-resistant cultivars will provide the IPM programs with insect resistant germplasm around which to build a more stable pest management program. North Carolina. The Peanut CRSP will provide funds to fill the gap in needed support to refine insect thresholds, select and develop pest resistant peanuts and develop an improved IPM program for North Carolina that will be economically and environmentally sound. This research will enhance the current research that coincides and compliments the objectives of the Peanut CRSP.

#### OBJECTIVES

1. To evaluate an international collection of peanut germplasm for resistance to a complex of insects in cooperation with Dr. J. C. Wynne (NCS/BCP/TP) Breeder, North Carolina State University and collaborators in Thailand and the Philippines.
2. Determine the damage potential of specific insects and the insect damage/plant phenological relationship (population dynamics) of important insects.
3. Study biology and ecology of important insects.
4. Determine the effect of cultural practices (planting date, seeding rate, row spacing, no-till, irrigation, fertilization, intercropping) on the insect population and damage to principal cultivars.
5. To establish insect/damage thresholds for the most important pests.
6. To develop a pilot pest management system that will incorporate information from the Peanut CRSP into existing peanut management systems in Thailand, Philippines and North Carolina.

#### ORGANIZATION

- |                                    |   |
|------------------------------------|---|
| A. <b>US Lead Institution:</b>     | North Carolina State University   |
| Principal Investigator:            | Dr. W. V. Campbell, Dept. of Entomology   |
| Cooperators:                       | Dr. J. C. Wynne, Department of Crop Science   |
| Technician:                        | Mr. Royce Batts, Department of Entomology   |
|                                    |   |
| B. <b>Counterpart Institution:</b> | Khon Kaen University, Khon Kaen, Thailand   |
| Principal Investigator:            | Dr. Manochai Keerati-Kasikorn, Department of Entomology   |
| Technicians:                       | Mr. Preecha Singha, Department of Entomology<br>Mr. Sungworm Malthong, Department of Entomology |

- C. **Counterpart Institution:** Department of Agriculture, Field Crops Bangkok, Thailand  
**Principal Investigator:** Dr. Sathorn Sirisingh, Entomology and Zoology Division  
**Cooperator:** Ms. Srisanom Pitak, Entomology and Zoology Division  
**Cooperator:** Mr. Pisit Sepswasdi, Entomology and Zoology Division  
**Technician:** Mr. Punya Pooksoon, Entomology and Zoology Division
- D. **Counterpart Institution:** University of the Philippines at Los Banos, Philippines  
**Principal Investigator:** Dr. Virginia Ocampo, Department of Entomology  
**Cooperator:** Dr. Candida Adalla, Department of Entomology  
**Cooperator:** M. R. V. Eborra, National Institute of Biotechnology  
**Cooperator:** Mr. D. R. Santiago, Department of Entomology  
**Cooperator:** Dr. Edwin Benigno, National Crop Protection Center  
**Technician:** Ms. Celia Medina, Mr. E. V. Santiago,  
 Mr. M. V. Navasero—Department of Entomology
- E. **USAID Project Officers:** Mr. Douglas Clark/Bangkok, Thailand  
 Dr. James Brady/Manila, Philippines

### TRAINING OUTPUTS

#### A. Degree Training

<u>Surname</u>	<u>Sex</u>	<u>University</u>	<u>Department</u>	<u>Degree</u>	<u>Date Degree Received</u>	<u>CRSP Support</u>
U.S. Citizens:						
Browde	M	NCSU	Entomology	MS		Partial
Thailand Citizen:						
Satayavirut	F	NCSU	Entomology	Ph.D.		Partial

#### B. Non-Degree Training

<u>Surname</u>	<u>Sex</u>	<u>Affiliation</u>	<u>Training</u>	<u>Location</u>	<u>Duration</u>
Thailand Citizen:					
Sathorn, Sirisingh	M	DOA	IPM	N. C.	3 weeks

**Training (National).** One graduate student from Thailand and one graduate student from the United States received training in insect pest management at North Carolina State University with Peanut CRSP funds during this report period. Mr. Browde completed his M. S. degree in Entomology in 1987. His thesis is the cost/benefit of selected insect management programs for peanuts. Ms. Turnjit Satayavirut is studying the tobacco thrips to refine the action threshold. Her thesis title will be the effect of thrips population and thrips damage on yield of principal cultivars grown in North Carolina. She will complete her studies in 1988.

**Training (International).** Dr. Sathorn Sirisingh, Department of Agriculture (DOA), Entomology and Zoology Division, attended the American Peanut Research and Educational Society (APRES) Annual Meeting at Orlando, Florida and presented a paper on research sponsored by CRSP. He also participated in research at the Peanut Belt Research Station at Lewiston, North Carolina for on-the-job training in insect pest management.

**Approach.** Tests will be conducted in areas representative of the peanut growing region for the country. Sites will be selected where specific insects are generally endemic. Tests will be established in the rainfed and dry-land crop.

In the Philippines *Trichogramma* egg parasite has potential for biological control of *Heliothis*, *Spodoptera* and other lepidopterous pests of peanuts. They may be reared and released in large number. The microbials *Bacillus thuringiensis* and nuclearpolyhedrosis virus may be propagated in the laboratory for use in the field. Insect resistant cultivars will be incorporated into the IPM package and evaluated against standard local cultivar for yield/loss relationships. Decision for "as needed" pesticides application in IPM will be based on thresholds established in the Philippines or CRSP thresholds for specific pests. It would be desirable to establish IPM tests in several areas for pest diversity and cropping system diversity.

In Thailand emphasis may be placed on management of leaf miner, thrips management as a vector of peanut yellow spot virus (PYSV), potato leafhopper, and the soil anthropod pests, the subterranean ant *Dorylus orientalis* and the white grub *Maladera* sp. (Coleoptera: Scarabaeidae). Peanut yellow spot virus (PYSV) and leaf miner are more prevalent in Northeast Thailand while the white grub is more endemic in north and northeast Thailand. Thrips and leafhopper occur more generally throughout Thailand. Tests will be established in areas where the insect problems exist. Uniform nurseries will be established in several areas to retest promising pest resistant germplasm lines. Pilot IPM tests that include pest resistant genotypes will be established in several regions to test the yield/damage loss relationship where insect resistant genotypes will be compared with local peanut cultivars. Decisions for "as needed" pesticide application will be based on thresholds established in Thailand supplemented with CRSP established insect/damage thresholds.

Pheromones will be used in sticky traps in North Carolina to relate adult trap catch with larval pod damage by the southern corn rootworm. Information will be used to establish a threshold for IPM. Pheromone shelf life and field weathering will be investigated for stability and longevity of the sex attracting pheromone. Uniform insect resistance nursery data will be collected from cooperators and summarized for publication. Insect pest management studies included prevention, "as needed" and untreated plots to verify the North Carolina action threshold used for decision making. Peanut genotypes will be incorporated into a separate IPM to determine the contribution of insect resistant germplasm in an IPM package.

#### ACCOMPLISHMENTS IN DETAIL

**Thailand.** Experiments were established in Chiang Mai and Mahasarakarm to determine the relationship between thrips population, thrips damage and yield. Thrips population was too low at Chiang Mai to affect yield. Thrips were effectively controlled with low rates of monocrotophos at Mahasarakarm. Monocrotophos applied at 45 and 60 days after planting resulted in significant yield increase (Table 1). Minimum insecticide rates and timing of application are now established for use in the IPM program for thrips control and management of peanut yellow spot virus (PYSV) by control of *Scirtothrips dorsalis*, the thrips vector of PYSV.

The subterranean ant is the most important and widespread soil pest in Thailand where it bores into developing peanut pods. Previous research has shown the subterranean ant is not found in peanut fields until plants are eight weeks old. When plants were sampled at week 6 to week 14 at Khon Kaen, plants were first infested at week 9 (Table 2). These data confirm previous sampling data and provide important information for management of subterranean ants.

The white grub *Maladera* sp. is another pod damaging soil pest of peanut. It is important to know the distribution pattern of the white grub; therefore plots were sampled at two harvesting dates. On October 1, 185 grubs were collected/plot and 75 pods/plot were damaged. When the plots were harvested on October 14, pods damage by the grub increased to 481 damaged pods (Figure 1). These data suggest early harvest will reduce pod damage.

Genotypes from the international test were retested at several locations in Thailand. Damage ratings were made for thrips, leafhopper, leaf miner, yellow spot virus and damaged pods. The standard local cultivars Tainan 9 and SK 38 exhibited more damage from the pest complex than selected genotypes. Root 31-1X NC 2214 (entry 26) and TG-1 X GP-NC 343 (entry 32) had less damage from all pests than the standard check (Table 3). All entries in this test yielded better than SK 38 while TG-1 X GP-NC 343 yielded approximately 30% more peanuts than Tainan 9 (Table 4). Thirteen genotypes

exhibited less damage from the leaf miner *Aproaema mordicella* than the susceptible cultivar Tainan 9 (Table 5).

Crop loss assessment for major insects of peanut was investigated at Rayong. Plots were treated to maintain insect damage-free plants. Other plots were not treated to build up insects and insect damage. Plots were sampled weekly starting June 29 and ending October 15. Results show unfilled pods were related to leafhopper and leaf miner damaged leaves at the  $R_4$  and  $R_5$  plant phenological stages. Seed yield was affected by leaf miner damage at  $R_2$  and especially by thrips damage at  $R_5$  stage (Table 6).

**Philippines.** Insect population, yield loss relationship was investigated for the cutworm *Spodoptera litura* at different plant phenological stages. Seed weight loss was highest when plants were infested at the pod development stage with 10 larvae/plant (Table 7). Economic threshold levels (ETL) developed for the cutworm did not reduce yield significantly for any phenological stage compared with the untreated check.

*Trichogramma* are important egg parasites of lepidopterous pests. *T. evanescens*, *T. chilonis* and *T. japonicum* reduced defoliation and increased yield compared with the untreated check.

Nucleopolyhedrosis virus (NPV) is effective for control of *Spodoptera litura* larvae. A dosage of  $1.73 \times 10^9$  polyhedral inclusion bodies resulted in 98% mortality of third instar *S. litura*. In excess of 70% mortality was obtained with a dosage of  $1.39 \times 10^7$  (Figure 2). Temperature affects the efficacy of NPV. Percent mortality with NPV exceeded 70% for temperatures between 10°C and 50°C. NPV was ineffective at temperatures above 70°C (Table 8).

*Bacillus thuringiensis* (B.t.) like NPV is another microbial used for control of lepidopterous pests. Strain differences were reported in the 1986 report. Two highly effective strains of B.t. gave 100% mortality on the day of application but mortality decreased over time. Four days after application B.t. was relatively ineffective against third instar larvae of *Spodoptera* (Table 9). Application of B.t. needs to coincide with an active population of young larvae since the half life is about two days. *Bacillus thuringiensis* is deactivated by some pesticides such as Eudosulfan. All insecticides at the standard recommended rate will reduce the effectiveness of B.t. when tank mixed. To reduce the loss of activity of B.t. it is necessary to reduce insecticide rates in tank mixes (Table 10).

**North Carolina.** The pheromone 10-methyl-2-tridecanone is highly effective in monitoring the adult southern corn rootworm (SCR). Pheromone trap catches averaged 504 per trap on August 3 while 40 unbaited sticky traps collected only 217 (Figure 3). Pheromone trap records also provided information on early season populations of SCR for decision making. Unbaited trap catches are too low for decision making except in late July and early August. Pheromone trap collection records were made weekly at four locations in 1987. Peak trap catches were recorded for August 3, 10 and 17. The relationship between trap catch and pod damage from the SCR is shown in Table 11. When early season trap catches (4 week period in June) averages more than 100 adult SCR then damage to pods is economic and insecticide application would be profitable. Additional confirmation of this preliminary threshold is necessary.

The international collection of germplasm that totaled 250 entries and was screened in North Carolina, Philippines and Thailand was reduced genotypes and retested in Thailand and North Carolina in 1987. The retest consisted of 31 entries ( $F_4$  Test), 44 entries (ICRISAT Test) and 79 entries (IGT Test). Thrips damage in the  $F_4$  for the two locations in Thailand coincided well (Table 12).

NC 6, NC 7 x GP-NC 343 and UPLPN 4 x NC 15729 had consistently lower damage in all tests. Leafhopper damage in the  $F_4$  test was low generally because of the high number of leafhopper resistant entries. This was especially evident in the North Carolina data (Table 13). NC 7 x GP-NC 343, NC 6, SK 38 x NC 10272, NC 7 x NC 10247, UPLPN 4 x NC 15729 and UPLPN 4 x NC 10247 exhibited low hopperburn from potato leafhopper in North Carolina and Thailand tests.

Thrips reduction in damage in the ICRISAT test ranged from 50 to 75% less for the more resistant genotypes compared with the standard cultivar susceptible check. Robut 33-1 x NC 2214, TG-1 x

GP-NC 343, Mani Pintar x (Robut 33-1 x NC 2232), Manfredi 68 x GP-NC 343, NC 1107 x (NC 2232 x NC 2214) and (NC 2232 x NC 2214) x TG 17 had consistently low thrips damage in North Carolina and Thailand. High levels of leafhopper resistance is present among the ICRISAT genotypes. Reduction in hopperburn among the most resistant entries ranged from 75% to 98% in the North Carolina test and 75% to 90% for the two tests in Thailand compared with local susceptible cultivars (Table 14).

The IGT Test contains genotypes with multiple pest resistance. Thrips damage was generally lower in the Thailand #1 test and generally higher damage was recorded for the North Carolina test. Genotypes with the lowest damage from thrips at all locations include NC 6, GP-NC 343, 10-P10-B1-B1-B1-B1-B2, Fx[Var 2750 x PI 259747]F2-B2-B1-B2-B1, NC 6 x NC 3033, (Gp-NC 343 x NC 5) x UF 70115, GP-NC 343 x NC5, FESR 1-P10-P2-B2-B1-B1-B2 and PI 467307 (Table 15). North Carolina entries had 65% to 75% less thrips damage than the susceptible cultivar Florigiant. Thailand test #2 entries had 85% to 95% less thrips damage than Tainan 9. Genotypes in the IGT test with low thrips damage also had low leafhopper hopperburn compared with Florigiant, SK 38 and Tainan 9 susceptible cultivars.

Peanut genotypes that had shown resistance or susceptibility to preharvest insects in the field were tested in the laboratory for resistance to post harvest moth complex, Indian meal moth and almond moth. The selected 39 entries were infested with laboratory-reared moths for preferential egg laying, larval survival and damage. Genotypes with lower numbers of surviving larvae and low feeding damage include NC 6, Robut 33-1 x NC 2214, J11 x (M 13 x NC 2232), NC 1107 x (NC 2232 x NC 2214), GP-NC 343, GP-NC 343 x NC 5, GP-NC 343 x NC 17367, 10-P10-B1-B1-B1-B1-B2 and PI 467307 (Table 16). The correlation of preharvest and post harvest pest resistance suggests genotypes have possible volatiles or secondary compounds that the plant and seed have in common that impart susceptibility or resistance to insects.

#### TRAVEL

**Host County (Thailand).** Dr. Sathorn Sirisingh, Department of Agriculture, Entomology Division, participated in research in North Carolina with W. V. Campbell. He also attended the APRES meeting in Orlando, Florida and presented a paper on CRSP supported research. This visit was for the period July 2 to July 27, 1987.

**United States (W. V. Campbell).** I attended and participated in APRES meeting in Orlando, Florida July 14-17, 1987.

W. V. Campbell visited collaborators Dr. Sathorn Sirisingh and Dr. Manochai Keerati-Kasikorn in Thailand and assisted in the evaluation of peanut genotypes for insect resistance (January 4 to February 7, 1988).

I attended and participated in the 8th Biennial Plant Resistance to Insects Workshop at Pacific Grove, California from February 27 to March 3, 1988.

#### PRESENTATIONS

Campbell, W.V., J.C. Wynne, M. Keekati-Kasikorn, S. Sirisingh, C. Adalla and E.P. Cadapan. 1987. Resistance of an international collection of peanut genotypes to insects in North Carolina, Philippines and Thailand. American Peanut Research and Education Society. 19:14.

Sirisingh, S. and S. Pitak. 1987. Effect of monocrotophos on thrips population, yellow spot virus and peanut yield. American Peanut Research and Education Society. 19:18.

#### PUBLICATIONS

Campbell, W.V. and W. Reed. 1987. Limits imposed by biological factors: pests. *In*, Food legume improvement for Asian farming systems (ed. E.S. Wallis and D.E. Byth). Proceedings of International Workshop, Khon, Kaen, Thailand, 1-5 September, 1986. ACIAR Proceeding No. 18:128-137.

## PLANS FOR 1988-1989

## Thailand

## 1. Department of Agriculture

- 1.1 Screening of groundnut lines for insect resistances.
- 1.2 An assessment of yield loss of groundnut due to thrips and leafhopper.
  - a. Identification
  - b. Sampling technique
  - c. Disease transmission
- 1.3 Study on measures for controlling subterranean ant and white grubs.
- 1.4 Study on the use of pheromone traps to monitor insect pests of groundnut.
- 1.5 Study on insect pest management of groundnut.

## 2. Khon Kaen University

- 2.1 Evaluation of groundnut lines for insect resistances.
- 2.2 Study on relationship between thrip damage and micronutrient deficiency in soil in the greenhouse.
- 2.3 Study on the implementation of insect pest management of groundnut based on the results of previous studies.
- 2.4 Monitoring of groundnut insects and their damage in farmers' fields.
- 2.5 Assessment of the population of bollworm *Heliothis armigera* by pheromone traps.

## Philippines

1. Test microbials for insect control for inclusion in peanut IPM.
2. Rear and release *Trichogramma* egg parasites and assess their potential for peanut IPM.
3. Establish pilot pest management tests in several areas in the Philippines.
4. Evaluate the benefit of incorporation of insect resistant genotypes into the existing IPM program compared with local cultivars.

## North Carolina

1. Retest and select genotypes from the international germplasm collection with multiple insect resistance.
2. Select multiple insect resistant genotypes for a Uniform Insect Resistance Nursery and distribute seed to cooperators in Georgia, Florida, Alabama and Virginia.
3. Use pheromone traps to monitor adult rootworm population and use the trap catch information to confirm preliminary thresholds.
4. Retest thresholds for confirmation and refinement for thrips and leafhopper.
5. Develop a pilot IPM program for assessment of insect resistant genotypes in a pest management program. Yield differences will be the basis for genotype differences and correct action threshold decisions.

Table 1. Thrips population on peanut sprayed with monocrotophos at different growth stages and peanut yield, Maharakarn, Thailand, 1987.

Plant ages <sup>1</sup>	No. of thrips/80 leaflets (1 day before and after application) <sup>2</sup>								Pod Yield	
	1st application		2nd application		3rd application		4th application		Avg.	kg/ha
	Before	After	Before	After	Before	After	Before	After		
1. 45,60,75,90	103.0	* 8.4	2.4	* 2.6	27.2	* 1.2	15.8	* 5.6	20.8	1,869 ab
2. 45,60,-,-	110.0	*10.0	3.6	* 1.0	28.8	19.0	28.6	21.4	27.8	2,033 a
3. -,-,75,90	71.0	60.6	29.8	40.0	48.4	* 0.8	13.0	* 1.4	33.1	1,564 ab
4. 45,-,75,-	106.8	*11.4	1.6	7.2	43.4	* 1.0	7.2	5.6	23.0	1,801 ab
5. -,60,-,90	77.0	79.2	32.8	* 1.2	13.4	10.0	21.4	* 2.8	29.8	1,514 ab
6. -,-,-,-	74.2	64.6	41.0	35.0	33.8	37.6	48.4	38.2	46.6	1,368 b

<sup>1</sup>Peanut plots were applied with 1% monocrotophos at different ages after planting.

<sup>2</sup>Asterisk in front of the number indicates treatment received insecticide application.



**Table 2. Mean numbers of infested plants/subplot caused by subterranean ants in the timing of infestation trial, 1987 rainy season, Ban Loop Yakha, Khon Kaen, Thailand.**

Plant age (week)	Mean no. of infested plants/subplot
6	0.0
7	0.0
8	0.0
9	27.0
10	27.0
11	34.0*
12	20.0*
13	32.0*
14	29.3*

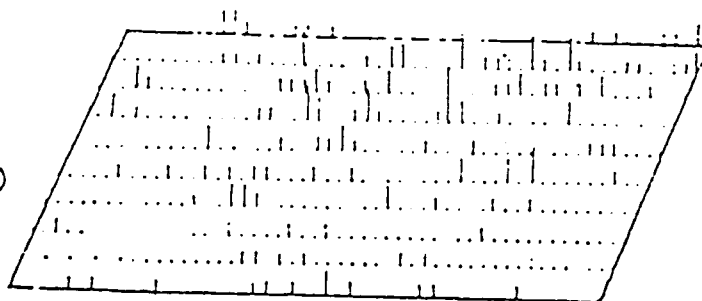
\* Includes damage caused by other soil arthropods such as millipedes

Harvesting date

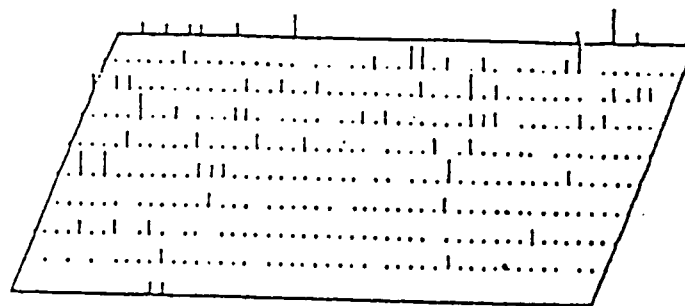
Distribution of Maladera sp.

Number of damaged pod

-Oct.1,1987  
(Field # 1)

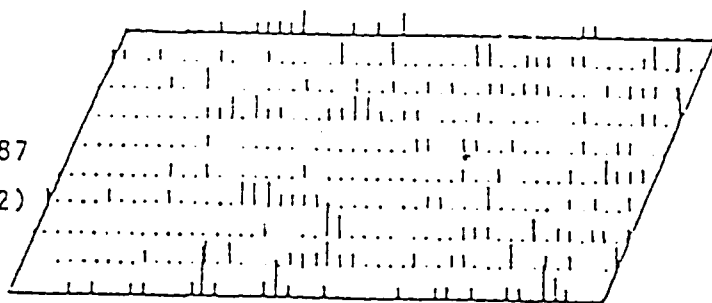


Max 5 insects/hill

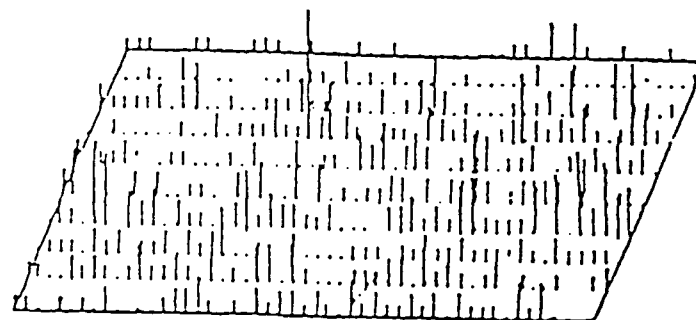


Max 3 pods/hill

-Oct.14,1987  
(Field # 2)



Max 4 insects/hill



Max 11 pods/hill

Fig.1 Distribution of Maladera sp. (Coleoptera:Scarabaeidae) and damaged pods in 5 x 10 m. peanut fields. Each line represents the average number of insect per hill. Khon Kaen,1987.

**Table 3. Resistance of breeding lines to insects in Thailand, 1987.**

Entry	Identity	No. of damaged leaflets <sup>1</sup>			yellow leaf	Damage Pods	
		LM	Thrips	LH	spot <sup>2</sup>	no.	%
<b>ICRISAT</b>							
6	(DH 3-20 x USA-20) x NC Ac 2232	29	27	18	6	8	5
19	Robut 33-1 x NC Ac 2214	23	25	22	6	8	4
26	Robut 33-1 x NC Ac 2214	12	14	27	7	9	7
32	TG - 1 x NC Ac 345	15	18	16	6	8	6
34	Manipinter x (Robut 33-1 x NC Ac 2232)	16	19	10	7	17	14
35	Manipinter x (Robut 33-1 x NC Ac 2232)	10	33	19	7	11	8
40	Manfredi - 68 x NC Ac 343	16	18	42	6	8	5
44	Makulured x NC Ac 343	22	19	13	6	9	6
49	Manfredi - 68 x NC Ac 343	26	30	17	7	21	16
58	Shulamit x (Robut 33-1 x NC Ac 2232)	13	21	17	7	6	6
70	(Goldin-1 x Faizpur 1-5) x NC Ac 2232)	19	51	26	7	10	27
71	(Goldin-1 x Faizpur 1-5) x NC Ac 2232)	20	22	12	7	5	14
	Tainan no. 9	29	23	43	7	8	25
	SK 38	31	39	45	8	15	21
<b>Insect Germplasm Test</b>							
9	GP-NC 343 x NC 17367	18	22	34	7	5	4
33	NC 17367 x GK-53	13	15	31	7	7	8
55	Nangai	36	21	48	7	20	21
	Tainan 9	23	20	45	8	13	11
	SK 38	27	21	40	8	32	27

<sup>1</sup>Counted from 160 leaflets,

LM = leafminer, LH leafhopper

<sup>2</sup>Rating score from 0-10

Table 4. Yield of peanut breeding lines in Thailand, 1987.

Entry	Identity	Harvesting hills	100 Seed weight	Pod Yield g/5m. row
<b>ICRISAT</b>				
6	(DH 3-20 x USA-20) x NC Ac 2232	19	25	257
19	Robut 33-1 x NC Ac 2214	15	32	198
26	Robut 33-1 x NC Ac 2214	17	32	211
32	TG - 1 x NC Ac 343	22	34	366
34	Manipinter x (Robut 33-1x NC Ac 2232)	21	27	281
35	Manipinter x (Robut 33-1x NC Ac 2232)	20	28	251
40	Manfredi - 68 x NC Ac 343	14	32	202
44	Makulured x NC Ac 343	14	30	247
49	Manfredi - 68 x NC Ac 343	19	34	249
58	Shulamit x (Robut 33-1x NC Ac 2232)	17	36	235
70	(Goldin-1 x Faizpur 1-5) x NC Ac 2232)	23	31	281
71	(Goldin-1 x Faizpur 1-5) x NC Ac 2232)	23	23	286
	Tainan no. 9	24	39	249
	SK 38	20	33	130
<b>Insect Germplasm Test</b>				
9	GP-NC 343 x NC 17367	22	32	253
33	NC 17367 x GK-53	23	41	250
55	Nangai	23	38	241
	Tainan 9	23	37	254
	SK 38	22	36	191

**Table 5. Groundnut lines with leaf damage caused by leafminer (*Proarema modicella*) less than Tainan 9, 1961 rainy season, Khon Kaen University, Thailand.**

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**F<sub>1</sub> Leafhopper test**

1. NC 7 x NC 302
2. SK 38 x GP NC 342
3. SK 38 x GP NC 343
4. UPL PN 4 x GP NC 343

**ICRISAT test**

1. DH 3-20 x NC Ac 2214
2. M-13 x NC Ac 2214
3. NC 17 x GP NC 343
4. Manipinter x (Robut 33-1 x NC Ac 2232)
5. GP NC 343

**Insect Germplasm test**

1. 10-P10-B1-B1-B1-B2
  2. GP NC 343 x NC 5
  3. NC 6 x NC 3033
  4. PI 459098
-

**Table 6. Prediction equations for unfilled pod/plant and for seed yield/plant of groundnut derived from a loss assessment trial in Rayong in 1987. Thailand.**

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Unfilled pod

$$Y = -0.0486 + 0.0244x_1^{**} - 0.36x_2^* + 0.5x_3^* \\ - 0.08x_4^* + 0.032x_5^*$$

$$R^2 = 0.2480$$

$$F = 6.60^{**}$$

Where  $X_1$  = No. of leaves per plant at  $R_2$

$X_2$  = % leaves damaged by leaf miner per plant at  $R_2$

$X_3$  = % leaves damaged by leafhopper per plant at  $R_4$

$X_4$  = % leaves damaged by leafhopper per plant at  $R_5$

$X_5$  = % leaves damaged by leaf miner per plant at  $R_7$

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Seed yield

$$Y = 0.772 + 0.011x_1^{**} + 0.02x_2^{**} + 0.114x_3^* \\ - 0.024x_4^{**}$$

$$R^2 = 0.3720$$

$$F = 14.959^{**}$$

Where  $X_1$  = No leaves per plant at  $R_1$

$X_2$  = No leaves per plant at  $R_2$

$X_3$  = % leaves damaged by leaf miner per plant at  $R_2$

$X_4$  = % leaves damaged by thrips per plant at  $R_5$

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\*  $P > 0.05$

\*\*  $P > 0.01$

**Table 7. Effects of cutworm *Spodoptera litura* population and plant phenological stage at time of infestation on seed yield of peanut line BPI P9. Los Banos, Philippines.**

Treatment larvae/plant	Plant stage	Mean seed weight a/ (tons/ha)
1	vegetative	3.276 abc
2 (ETL) <sup>b</sup>	vegetative	2.375 d
3	vegetative	2.348 d
1	pod development	2.736 d
5 (ETL) <sup>b</sup>	pod development	2.360 d
10	pod development	1.976 e
3	full seed development	3.576 ab
8 (ETL) <sup>b</sup>	full seed development	2.824 cd
10	full seed development	2.456 d
2	all season	2.452 d
0	insecticide check	3.680 a
0	untreated check	2.980 bcd

<sup>a</sup> Means followed by the same letter are not significantly different by Duncans Multiple Range Test (DMRT=.05)

<sup>b</sup> Economic threshold level established for cutworm larvae/plant

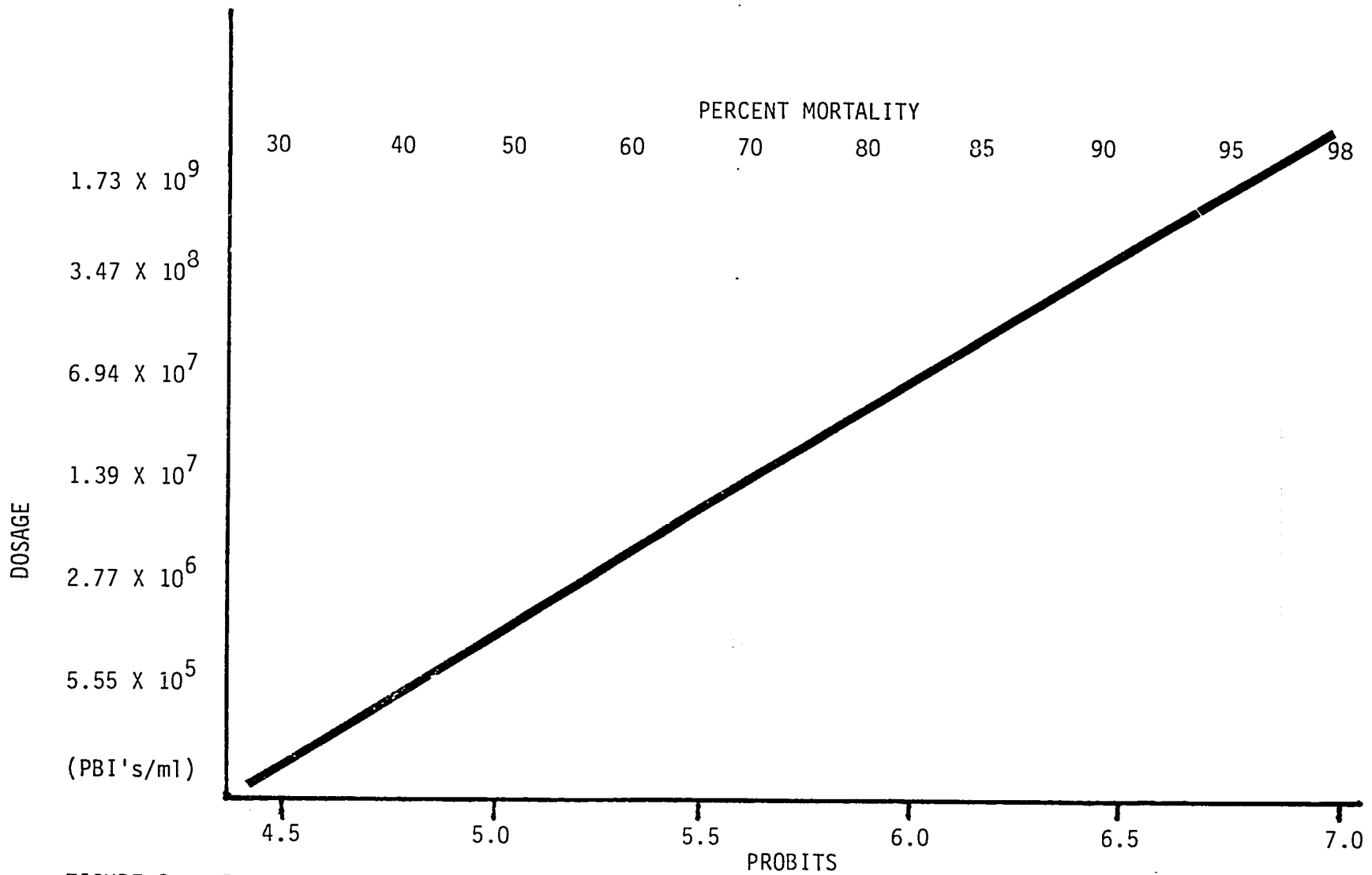


FIGURE 2. Dosage-mortality response curve of Nuclearpolyhedrosis virus (NPV) against third instar larvae *Spodoptera litura* 7 days after treatment. UPLB, Los Banos, Philippines



**Table 8. Effect of different temperatures on the virulence of nuclearpolyhedrosis virus (NPV) on cutworm larvae. University of Philippines, Los Banos, Philippines.**

Treatment <sup>a</sup> °C	% Infection <sup>b</sup>	% Mortality <sup>b</sup>
10	100	78
20	100	70
30	100	84
40	100	74
50	98	70
60	80	62
70	32	4
80	8	0
90	0	0
100	0	0

<sup>a</sup> Crude extract ( $8.67 \times 10^7$  PIB's/ml) subjected to the different temperatures for 15 minutes.

<sup>b</sup> From fifty 3rd instar larvae (10 replicates, 5 larvae/replicate) reading taken 7 days after treatment.

**Table 9. Field persistence of two *Bacillus thuringiensis* strains applied to peanut leaves<sup>a</sup>.  
University of Philippines, Los Banos, Philippines.**

Day after application	% Mortality <sup>b</sup>	
	Strain B23	Strain B25
0	100	100
1	70	84
2	58	60
3	36	48
4	10	25
5	0	9
6	0	5
7	0	0

<sup>a</sup> *B. thuringiensis* powder was sprayed to the underside of the peanut leaves at

<sup>b</sup> Third instar larvae used as test insect.

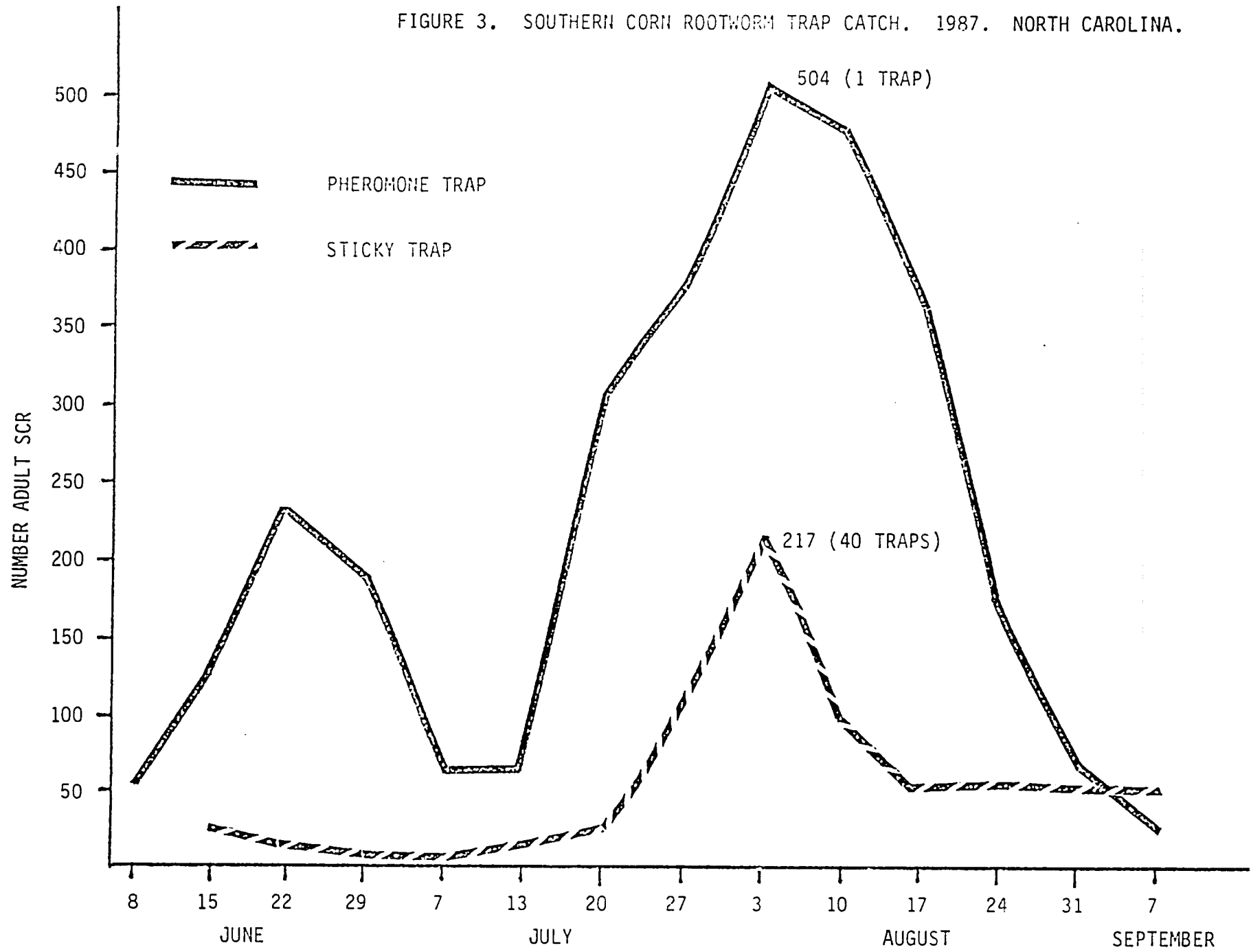
Table 10. Compatability of *Bacillus thuringiensis* strains with different chemical insecticides<sup>a</sup>.  
University of Philippines, Los Banos, Philippines.

Insecticide	Dose <sup>b</sup>	No. <i>B. thuringiensis</i> colonies	
		Strain B23	Strain B25
Endosulfan	R	0	0
	½ R	0	0
	¼ R	0	0
Monocrotophos	R	0	0
	½ R	30	40
	¼ R	24	42
Deltamethrin	R	25	50
	½ R	26	39
	¼ R	26	45
Triazophos	R	0	0
	½ R	14	20
	¼ R	25	42
Malathion	R	4	16
	½ R	30	38
	¼ R	35	41
Control (1% Hexane)		34	46

<sup>a</sup> 10<sup>7</sup> spore concentration of *B. thuringiensis* was suspended in the test chemical for 30 minutes

<sup>b</sup> R = recommended rate

FIGURE 3. SOUTHERN CORN ROOTWORM TRAP CATCH. 1987. NORTH CAROLINA.



**Table 11. Relationship between pheromone trap catch during June and southern corn rootworm (SCR) pod damage. North Carolina.**

Location	Avg. No. SCR collected/trap <sup>a</sup>	Avg. % pod damaged <sup>b</sup>
1987		
Bertie 1	200	43
Bertie 2	192	35
Halifax	20	3
Rocky MT	170	67
1986		
Bertie 1	120	5
Bertie 2	70	3
Halifax	38	1
Rocky MT	116	13
1985		
Bertie 1	121	25
Bertie 2	176	16
Halifax	93	3
Rocky MT	88	0

<sup>a</sup> Average number per trap for a 4-week period during June.

<sup>b</sup> Based on average of 5-plant samples in September.

Table 12. Differences among peanut genotypes in thrips damage. F<sub>4</sub> Test. North Carolina and Thailand, 1987.

Entry No. LH	ID	Thrips damaged leaves			
		N.C.	Thailand		Avg.
			#1	#2	
1	NC 7 x GP-NC 343	28.0	21.5	11.7	16.6
3	NC 7 x NC 302	47.5	22.5	14.5	18.5
4	NC 7 x NC 15745	31.2	22.2	19.7	20.9
5	NC 7 x NC 10247	40.0	36.5	28.7	32.6
6	NC 7 x NC 10272	37.0	44.0	25.5	34.7
7	SK 38 x GP-NC 343	37.5	27.2	27.2	27.2
8	SK 38 x NC 342	31.2	25.0	19.0	22.0
12	SK 38 x NC 15729	48.7	29.7	26.0	27.8
13	SK 38 x NC 10272	62.5	33.0	34.5	33.7
15	Lampang x GP-NC 343	17.2	34.2	43.0	38.6
17	NC 6	14.7	13.5	11.5	12.0
19	Lampang x NC 15729	45.0	25.5	27.2	26.3
20	Lampang x NC 10247	38.7	53.0	43.2	48.1
22	Tainan 9 x GP-NC 343	31.2	27.5	38.0	32.7
23	Tainan 9 x NC 342	38.7	30.7	41.2	35.9
25	Tainan 9 x NC 302	38.7	32.0	51.0	41.5
28	Tainan 9 x NC 10247	39.5	56.2	64.5	60.3
31	CES 101 x NC 342	28.0	31.5	50.5	41.0
36	CES 101 x NC 10272	51.2	56.0	51.2	53.6
38	UPL Pn4 x GP-NC 343	21.7	39.0	11.2	25.1
40	UPL Pn4 x NC 15729	22.2	21.2	18.0	19.6
42	UPL Pn4 x NC 10247	30.0	60.5	14.0	37.2
43	NC 7	31.7	28.7	32.0	30.3
44	SK 38	33.7	38.5	57.0	47.7
45	Lampang	47.9	34.0	71.5	52.7
46	Tainan 9	26.2	55.5	93.7	74.6
49	GP-NC 343	14.2	11.7	10.0	10.8
52	Florigiant	56.2	--	--	--
51	NC 302	20.0	18.2	7.7	12.9
39	UPLPN4 x NC 302	--	31.2	9.7	20.4
--	Tainan 9	--	30.5	103.5	67.0

Table 13. Differences among peanut genotypes in leafhopper damage. F<sub>4</sub> Test. North Carolina and Thailand. 1987.

Entry No. LH	ID	Hopperburned leaves			
		N.C.	Thailand		Avg.
			#1	#2	
1	NC 7 x GP-NC 343	8.2	22.0	15.7	18.8
3	NC 7 x NC 302	14.7	20.2	14.7	17.4
4	NC 7 x NC 15745	11.2	26.7	46.0	36.3
5	NC 7 x NC 10247	11.2	21.5	9.0	15.2
6	NC 7 x NC 10272	10.5	26.5	25.2	25.8
7	SK 38 x GP-NC 343	22.2	28.0	12.0	20.0
8	SK 38 x NC 342	7.7	27.2	32.0	29.6
12	SK 38 x NC 15729	18.5	29.5	26.2	27.8
13	SK 38 x NC 10272	15.0	25.2	12.7	18.9
15	Lampang x GP-NC 343	10.5	46.0	63.2	54.6
17	NC 6	3.0	21.5	18.0	19.7
19	Lampang x NC 15729	17.2	30.2	34.0	32.1
20	Lampang x NC 10247	8.2	29.0	12.2	20.6
22	Tainan 9 x GP-NC 343	12.0	37.7	30.5	34.1
23	Tainan 9 x NC 342	13.5	24.7	52.0	38.3
25	Tainan 9 x NC 302	19.7	32.7	54.5	46.6
28	Tainan 9 x NC 10247	5.2	31.2	29.2	30.2
31	CES 101 x NC 342	16.2	24.7	34.0	29.3
36	CES 101 x NC 10272	14.2	28.7	50.2	39.4
38	UPL Pn4 x GP-NC 343	8.2	18.5	43.2	30.8
40	UPL Pn4 x NC 15729	9.2	18.0	16.7	17.3
42	UPL Pn4 x NC 10247	7.5	21.5	7.2	14.3
43	NC 7	9.5	25.5	17.0	21.2
44	SK 38	14.2	45.0	48.7	46.8
45	Lampang	35.0	45.7	52.2	48.9
46	Tainan 9	25.5	59.0	69.5	64.2
49	GP-NC 343	2.0	20.2	11.7	15.9
52	Florigiant	31.2	--	--	--
51	NC 302	10.0	13.7	4.0	8.8
39	UPLPN4 x NC 302	--	23.0	18.2	20.6
--	Tainan 9	--	57.5	63.5	60.5

**Table 14. Differences among peanut genotypes in leafhopper damage. ICRISAT Test. North Carolina and Thailand. 1987.**

Entry No. LH	Pedigree	Hopperburned leaves			
		N.C.	Thailand		Avg.
			#1	#2	
3	Manfredi 68 x GP-NC 343	22.5	28.5	35.7	32.1
4	DH 3-20 x NC Ac 2214	11.7	30.0	34.7	32.3
6	(DH 3-20 x USA-20) x NC 2232	6.5	17.7	7.0	12.3
8	(Robut 33-1 x NC 2821) x NC 2232	7.5	27.7	15.7	21.7
11	Robut 33-1 x GP-NC 343	11.0	18.0	11.5	14.7
13	Robut 33-1 x NC 2214	1.0	12.5	1.0	6.7
17	M-13 x NC 2214	13.0	25.2	13.7	19.4
18	Robut 33-1 x NC 2214	1.0	25.7	43.0	34.3
19	Robut 33-1 x NC 2214	24.0	22.2	44.2	33.2
21	Robut 33-1 x NC 2214	1.0	8.2	1.7	4.9
23	M 13 x NC 2214	11.2	19.2	19.7	19.4
24	M 13 x NC 2214	6.7	26.2	29.7	27.9
25	Robut 33-1 x NC 2214	23.7	21.2	30.0	25.6
26	Robut 33-1 x NC 2214	16.7	27.0	23.5	25.2
30	Manfredi 68 x GP-NC 343	23.0	25.5	19.0	22.2
31	NC 17 x GP-NC 343	15.7	16.5	28.0	22.2
32	TG-1 x GP-NC 343	20.5	15.5	7.0	11.2
33	G-120 x NC Ac 2232	13.0	22.5	10.0	16.2
34	Mani Pintar x (Robut 33-1 x NC Ac 2232)	1.5	10.0	5.0	7.5
35	Mani Pintar x (Robut 33-1 x NC Ac 2232)	2.7	18.5	8.5	13.5
37	TMV-10 x NC Ac 2232	9.0	28.2	40.5	34.3
39	28-206 (France) x NC Ac 10247	22.5	29.2	15.5	22.3
40	Manfredi 68 x GP-NC 343	37.5	42.2	55.0	48.6
41	X52-X-X-3-B x (M 13 x NC Ac 2214)	7.2	22.0	15.0	18.5



Table 14. (continued)

44	Makulur Red x GP-NC 343	48.7	13.0	16.7	14.8
45	Manfredi 68 x GP-NC 343	20.0	25.0	15.2	20.1
47	Manfredi 68 x GP-NC 343	18.2	25.5	29.5	27.5
49	Manfredi 68 x GP-NC 343	8.5	17.2	7.5	12.3
51	J11 x (M 13 x NC Ac 2232	1.0	9.5	0	4.7
54	(DH 3-20 x USA 20) x NC 2232	12.0	34.2	37.5	35.8
55	Goldin-1 x NC Ac 2232	11.0	25.7	15.7	20.7
56	Tifspan x NC Ac 2232	19.2	25.7	34.7	30.2
58	Shulamit x (Robot 33-1 x NC 2232)	2.5	17.0	10.0	13.5
62	NC 1107 x (NC 2232 x NC 2214)	1.2	18.5	2.5	10.5
63	NC 1107 x (NC 2232 x NC 2214)	1.2	15.0	8.0	11.5
67	(NC 2232 x NC 2214) x TG-17	2.7	14.2	10.7	12.4
70	(Goldin-1 x Faizpur 1-5) x NC 2232	10.0	26.2	24.7	25.4
71	(Goldin-1 x Faizpur 1-5) x NC 2232	2.2	12.2	7.5	9.8
72	(Goldin-1 x Faizpur 1-5) x NC 2232	3.0	16.5	21.7	19.1
73	NC 6	3.5	16.5	9.0	12.7
74	Florigiant	42.5	--	--	--
75	GP-NC 343	4.0	--	--	--
--	SK 38	--	34.7	46.0	40.3
--	Tainan 9	--	42.7	52.2	47.4

**Table 15. Differences among peanut genotypes in thrips damage. IGT Test. North Carolina and Thailand. 1987.**

Entry No. LH	ID	Thrips Damage		
		N.C.	Thailand	
			#1	#2
1	Early Bunch x NC 18016	50.0	15.7	16.2
2	Early Bunch x NC 18016	32.5	24.0	38.0
4	NC 17213 x NC 7	57.5	17.7	40.0
5	NC 6 x Early Bunch	41.2	14.7	16.2
6	NC 7 Select	41.7	12.7	13.7
8	GP-NC 343 x NC 17367	29.5	17.0	8.2
9	GP-NC 343 x NC 17367	33.7	21.5	12.7
12	2-P1-B1-B4-B1-B1-B2	25.0	12.7	5.2
13	10-P10-B1-B1-B1-B1-B2	14.7	11.5	4.7
14	3-P13-B2-P2-B2-B1-B1-B2	28.2	14.7	26.0
15	FESR 1-P10-P2-B2-B1-B1-B2	16.2	9.0	5.7
16	Fx[Var. 53-68 x PI 298115] F <sub>2</sub> -B3-B1-B1-B2-B1	40.0	9.7	31.7
17	Fx[Var. 55-437 x NC Ac 17090]F <sub>2</sub> -B2-B1-B2-B1	48.7	14.5	18.2
18	Fx[G37 x PI 259747]F <sub>2</sub> -B1-B1-B2-B1	29.5	22.5	16.0
19	Fx[Var. 2750 x PI 259747]F <sub>2</sub> -B2-B1-B2-B1	8.7	12.2	13.5
20	NC 2 x NC 5	29.2	10.0	34.0
21	NC 5 x GP-NC 343	26.2	7.5	8.0
22	NC 5 x GP-NC 343	37.0	18.7	7.7
23	Florigiant x GP-NC 343	30.5	3.5	14.0
24	Florigiant x GP-NC 343	35.0	15.7	11.0
25	GP-NC 343 x NC 7	35.0	14.2	12.0
26	GP-NC 343 x NC 7	24.5	21.7	4.7
27	GP-NC 343 x NC 5	19.5	9.7	7.7
28	GP-NC 343 x NC 5	28.7	9.5	5.7
29	GP-NC 343 x NC 5	28.2	13.5	17.2

Table 15. (continued)

30	NC 17969 x NC 18233	19.2	22.5	16.0
31	NC 6	18.7	10.2	5.0
32	GP-NC 343	16.7	9.2	2.5
33	NC 17367 x GK 53	31.2	14.7	45.5
34	NC 18017 x NC 18018	37.5	14.0	24.0
37	(GP-NC 343 x NC 5) x UF 70115	20.5	15.0	4.5
38	(MGS 9 x NC 17090)F <sub>2</sub> -B1-B2-B1	41.2	22.0	11.0
39	NC 6 x NC 3033	24.2	19.7	7.2
40	NC 6 x NC 3033	16.2	10.7	11.5
41	NC 6 x NC 3033	15.2	17.2	5.7
42	NC 6 x NC 3033	22.5	13.2	37.0
43	NC 6 x NC 3033	18.0	13.5	9.5
44	NC 6 x NC 3033	21.5	15.2	9.0
45	NC 6 x NC 3033	22.2	12.2	3.0
46	NC 6 x NC 3033	21.0	11.5	8.0
47	NC 6 x NC 3033	18.7	17.5	10.2
48	NC 6 x NC 3033	17.2	12.7	4.2
49	NC 6 x NC 3033	20.7	20.5	4.2
52	AH 3275	35.0	12.2	47.5
53	Bhairhwa Local	30.0	17.7	15.5
55	Nangai	19.2	20.7	125.0
64	Tachimasari	19.2	10.5	16.5
65	Valencia	31.2	14.7	69.5
66	Benihandach	43.7	7.7	12.2
68	Tanganica No. 4	50.0	18.0	8.2
70	Kanto No. 38	56.2	20.7	47.0
71	Barberton	42.5	10.5	29.2
72	MH 372 (Sudan)	46.2	21.0	7.7
73	MH 383 (Sudan)	41.2	23.0	21.7

Table 15. (continued)

76	Florigiant	57.5	24.7	31.7
77	Kintaki (Thailand)	43.7	30.0	69.0
83	Tainan No. 9	35.0	15.2	90.2
85	Tainan No. 6	46.2	9.5	70.5
89	Taiwan No. 9	52.5	23.0	92.0
91	Comet	18.7	16.0	17.5
95	Tamnut 74	16.7	14.7	29.2
99	69-101	38.0	16.2	11.7
100	73-30	41.2	29.0	29.5
109	PI 459095, Masashisennari	24.5	8.2	28.5
111	PI 459098, Toyokodachi	41.2	17.5	11.2
112	PI 459099, Wakaminori	38.2	17.5	14.7
113	PI 467307, Ben-yang Shang-Shro Dur	19.7	10.0	9.2
116	TMV 2	28.7	18.0	39.2
117	NC Ac 17133RF	36.2	18.2	51.5
121	Sunbelt Runner	35.0	16.0	60.0
123	NC 7	47.5	17.0	21.2
--	Florigiant	65.0	--	--
--	SK 38	--	20.0	55.7
--	Tainan 9	--	18.0	106.5

Table 16. Ovipositional preference and damage to shelled peanuts by Indian meal moth and almond moth, a post harvest pest complex. Laboratory Test. Raleigh, North Carolina.

Genotype entry	Identity	Avg. no. larvae	Avg. % damage
F <sub>4</sub> -1	NC 7 x GP-NC 343	118.7	4.5
F <sub>4</sub> -3	NC 7 x NC 302	199.7	19.5
F <sub>4</sub> -5	NC 7 x NC 10247	98.5	11.7
F <sub>4</sub> -7	SK 38 x NC 343	187.7	17.2
F <sub>4</sub> -17	NC 6	69.5	3.0
F <sub>4</sub> -28	Tainan 9 x NC 10247	123.5	14.7
F <sub>4</sub> -42	UPLPN4 x NC 10247	127.0	8.2
F <sub>4</sub> -49	GP-NC 343	135.5	5.5
F <sub>4</sub> -51	NC 302	137.2	6.2
F <sub>4</sub> -52	Florigiant	141.0	9.5
IC 13	Robut 33-1 x NC 2214	89.7	2.7
IC 18	Robut 33-1 x NC 2214	75.7	2.2
IC 21	Robut 33-1 x NC 2214	56.7	2.0
IC 34	Mani x (33-1 x NC 2232)	110.7	4.0
IC 35	Mani x (33-1 x NC 2232)	168.2	5.0
IC 41	x52-x-3-Bx (M 13 x 2214)	113.2	4.7
IC 51	J11 x (M 13 x NC 2232)	82.5	2.7
IC 62	NC 1107 x (2232 x 2214)	123.5	1.7
IC 63	NC 1107 x (2232 x 2214)	90.7	1.0
IC 67	(2232 x 2214) x TG 17	150.2	2.7
IC 71	(Goldins x Faizpur) x	135.5	5.5
IC 73	NC 6	93.2	4.0
IC 74	Florigiant	246.5	23.0
IC 75	GP-NC 343	73.7	3.0
IGT 1	Early Bunch x NC 18016	89.5	4.2
IGT 8	GP-NC 343 x NC 17367	61.0	3.0
IGT 13	10-P-10-B1-B1-B1-B1-B2	83.2	4.5
IGT 26	GP-NC 343 x NC 7	102.0	3.0
IGT 27	GP-NC 343 x NC 5	86.2	3.7
IGT 28	GP-NC 343 x NC 5	67.2	3.0
IGT 29	GP-NC 343 x NC 5	124.2	5.2
IGT 34	NC 18017 x NC 18018	156.0	13.2
IGT 32	GP-NC 343	127.0	3.5
IGT 113	PI 467307	92.0	4.5
IGT 42	NC 6 x NC 3033	104.0	5.7
IGT 19	F x [var. 2750 x PI 25747]F2	88.2	4.0
IGT 62	NC 6	81.2	3.2
IGT 123	NC 7	99.5	5.2
IGT 124	Florigiant	119.0	12.2

GA/PV/N,TP

**Peanut Viruses: Etiology, Epidemiology****and Nature of Resistance**

University of Georgia—Institute for Agricultural Research

at Ahmadu Bello University, Nigeria

James Demski, Principal Investigator, UGA

**INTRODUCTION**

Groundnut (peanut) rosette is a major constraint in the production of peanut in Africa, and along with other viruses causes significant yield losses. In addition to actual yield losses, the periodic occurrences of rosette epidemics, inducing local total crop loss, has farmers planting other more dependable crops. Before control measures can be implemented, the source of the rosette virus must be found and the nature of resistance elucidated and resistance incorporated into drought-resistant, short season peanuts.

Peanut stripe virus (PStV) was first discovered naturally infecting peanuts in the U.S. in 1982. After PStV was characterized and methods of identity developed, data from surveys in different countries of the world indicated that PStV is the most prevalent virus infecting peanut in S. E. Asia (Indonesia, Malaysia and the Philippines) and China. A recent report documented the occurrence of PStV in India. Thus PStV is prevalent in areas where a large majority of peanut production occurs. Even though PStV may induce only low yield losses, the virus infects a sufficient number of plants to have an economic impact on total peanut production.

The incidence of tomato spotted wilt virus infecting peanut has increased in the U.S. in the past decade and now threatens production in a three county area of Texas. Increased incidence in the eastern states has been noted, and because of high yield loss by the virus could pose a new constraint for peanut production.

**MAJOR ACCOMPLISHMENTS****Establishment of project**

This project was established when Dave Cummins and James Demski went to the Institute for Agricultural Research (Nigeria) in February 1982 to discuss goals, research objectives, and collaborative work with Director John Davies, Dr. Colin Harkness and Dr. Steve Misari. Mutual interests were confirmed and a Memorandum of Understanding and Plan of Work was signed.

In 1987, the project was expanded to include Thailand as a host country. Dr. Sopone Wongkaew at Khon Kaen University is the primary cooperator. A separate memorandum of understanding was not signed but support will be provided through an existing Peanut CRSP project.

**International travel**

In April 1988, Drs. Steve Misari and Okon Ansa traveled from Nigeria to London where they were met by Drs. Kuhn and Demski for a three day meeting to cover work completed and work planned. In June 1988, Phindile Olorunju returned to Nigeria to implement her field research studies towards the Ph.D. In August 1988, Kuhn and Demski traveled to Nigeria where they conferred with Olorunju on her research progress and worked with Misari and Ansa on cooperative studies.

**Research results**

Plants infected with groundnut rosette have two agents associated with them; the groundnut rosette virus (GRV) which is responsible for the symptom expression and the groundnut rosette assistor virus

(GRAV), when alone, is symptomless. However, recent studies indicate that when plants are infected with both GRV and GRAV the detrimental effect is greater than when the plants are infected with GRV alone.

Yearly surveys have shown that 1988 was another epidemic year for groundnut rosette in the Samaru-Zaria area of Nigeria. This outbreak coupled with outbreaks in 1983 and 1985 has discouraged farmers from producing peanuts.

Lines resistant to groundnut rosette were released in 1986 and these were increased in 1987. For the 1988 season, 20 tons of seed resistant to GRV were available to growers. However, because of large numbers of isolated small farms, most growers are still using susceptible lines.

Early maturing peanut lines 55-437 (Ex-Dakar), RRB, 48-115B and K879.78 that have been observed to be highly drought tolerant (rosette susceptible) and mature 95-110 days are recommended for the Sudan savanna zone and parts of the Northern Guinea savanna zone. Rosette resistant late maturing lines M554.76, RMP-12 and RMP-91 are recommended for Northern and Southern Guinea savanna zones.

Yield loss assessment for PStV in groundnuts in Thailand was only partially successful. Virus spread from inoculated to non-inoculated plants and by 80 days, all treatments had equal virus incidence. Never-the-less pod yields were reduced in 3 of 5 inoculated peanut lines and one line showed tolerance.

Purification procedures developed for one isolate of PStV may not be effective for purifying virus of a different isolate. However effective procedures have been developed for purification of all known isolates.

Tomato spotted wilt virus (TSWV) isolated from peanuts in the U. S. was purified and an antiserum produced. Little serological differences were found between isolates of TSWV from peanuts, tobacco, pepper or tomato in the U. S.

Data from comparison of TSWV from U.S. peanuts and TSWV from peanuts in India, using the immuno-blot technique, reveals a major difference in the virus from the two continents. Of the five polypeptides in the virions of the U. S. isolates, only two reacted with the Indian isolate antiserum. These isolates are considered strains of each other.

Direct comparison of different serological systems was made to detect PStV in peanut tissue or seed. The direct antigen coating (DAC) system was the most sensitive. The simplified rapid direct antigen coating of direct ELISA or the plate trapped antigen of the indirect ELISA could be completed in the shortest time. The penicillinase system is the cheapest to employ.

A virus isolated from *Desmodium* sp. (beggarweed) in an experimental peanut field in Tift Co., Georgia infects peanuts systemically causing systemic necrosis and stunting. The virus was identified as a new isolate of PStV.

Three distinct viruses can cause necrosis, stunting and yellowing on peanut which sometimes cannot be distinguished visually but must be confirmed by serology. These are TSWV, beggarweed isolate of PStV and the necrosis strain of peanut mottle virus (PMV).

Antisera to potato leaf roll virus and beet western yellows virus can detect the GRAV (symptomless virus) in rosetted plants in Africa. Using these same antisera, on U.S. peanuts, some positive reactions occurred. Transmission tests need to confirm the presence of a virus in U.S. peanuts that could be related to the GRAV in Africa.

#### EXPECTED IMPACT OF PROJECT

**In host-country.** Because of the epidemics of rosette in Nigeria in 1975 and 1985, many growers have reduced or eliminated peanut production in their farming operations. Resistance lines are available for the southern peanut growing zone of Nigeria. Expanded efforts to increase and

disseminate resistant lines should reverse the lower production trend. Breeding programs continue in Nigeria and success in releasing higher yielding resistant lines should provide the means for production increases. Control of rosette disease should permit growers to produce peanuts profitably and thus reverse the declining production trends and regain the per capita production.

**In Thailand.** S. E. Asia and China peanuts have the highest incidence of PStV in the world. Additionally the symptom expression in peanuts infected with PStV in S. E. Asia is quite severe. Preliminary data indicate that yield reduction in infected Asian peanuts may be higher than U. S. peanuts infected with PStV. Use of virus-free seed and other control measures should reduce the current yield loss in Asian peanut production.

**In U.S.** The CRSP virus project has led to the discovery of a new virus infecting peanut in the United States. This virus has the potential to be a damaging virus in U.S. peanut production if not controlled. Programs are underway to eliminate this seed-borne virus before it becomes endemic in other hosts that could serve as new sources of inoculum.

The University of Georgia will maintain an antiserum bank and a seed bank of virus-free seed. It will be possible to achieve rapid diagnosis of the peanut virus diseases in any part of the world without sophisticated facilities by serological tests and host reactions. These tools will be available on a world basis. If written instructions for diagnosis are inadequate, a short course will be developed for presentation wherever needed.

## GOAL

Virus diseases, in epidemic proportion, are limiting factors in peanut production. The three most destructive viruses infecting peanut, on a worldwide basis, are peanut mottle (PMV), groundnut rosette (GR), and bud necrosis (BN) caused by TSWV. PStV is increasing in importance because of expanded distribution and increased incidence. Based on current information our goal is to reduce virus disease losses by GRV through resistant lines being issued by a breeding program; by the use of virus-free seed for PStV with continued searching for resistance; by obtaining data on the epidemiology of TSWV and seeking virus and/or vector resistance; and by a general program of virus identification, characterization, strain relationships and transmission of the viruses by vectors. Therefore, the major goal of this project is through research efforts to attain a better understanding of the causal agents and virus diseases so that some methods of control can be developed for the different viruses in the different countries.

## OBJECTIVES

- A. Support a breeding program to develop and/or select groundnut rosette resistant lines adaptable to different geographic zones in Africa.
- B. Separate, identify and characterize the strains of PStV infecting peanuts in S. E. Asia.
- C. Initiate a program of producing virus-free seed adaptable to S. E. Asia conditions.
- D. Work cooperatively with ICRISAT personnel on unified research efforts on PStV and TSWV for disease control.
- E. Identify other peanut viruses, determine the variants of these agents, and develop means of rapid identification.

## Approach

At the Tulsa, Oklahoma meetings in July 1988 the Peanut CRSP Board of Directors recommended that this Virus Project shift emphasis from Nigeria to S. E. Asia. Support for the current Nigerian graduate student Phindile Olorunju will continue (expected completion December 1989).



- A. Mrs. Phindile Olorunju is working towards the Ph.D. at the University of Georgia but is doing her dissertation research in Nigeria. Using resistant, 'intermediate' resistant, and susceptible peanut lines (resistant to groundnut rosette),  $F_1$  and  $F_2$  crosses, and backcrosses are being made. Increased seed is being planted in Nigeria field plots where infected plants are providing heavy inoculum pressure. The purpose is to determine the inheritance of resistance and the reaction of peanut genotypes to the rosette causal agents. On site coordination in Nigeria is under Dr. S. Misari supervision with Drs. Kuhn and Demski as advisors.
- B. Dr. Sopone Wongkaew at Khon Kaen University in Thailand is collecting different isolates of PSTV from different areas of the world. Arrangements are being made for Sopone to take these isolates to a laboratory in France where he will directly compare all isolates under similar conditions and characterize them as to strains or isolates.
- C. A program to produce PSTV-free seed will be initiated in Thailand and possibly the Philippines. Peanut lines adaptable to the host countries will be planted in a greenhouse and rigorously assayed for PSTV. All infected plants will be rogued permitting only healthy seed to be produced.
- Areas of each host country are being sought where PSTV is not endemic. These may be areas where peanuts have never been grown but the climate will permit peanut production. In these areas the greenhouse virus-free seed will be increased.
- Virus-free seed will be planted in areas where PSTV has been a restrictive problem in the past to determine if the higher cost of virus-free seed will be economically beneficial.
- D. Maintain contact with Dr. D.V.R. Reddy, the principal virologist of the legumes program at ICRISAT, so that duplication of work is not done but coordination of work such as searching for PSTV resistance in peanuts and virus-free seed program enhance the regional peanut production.
- E. Peanuts in all host countries and the U.S. will continually be monitored for viruses. New isolates, strains or viruses will be characterized and appropriate methods for handling will be devised.

#### ORGANIZATION

- A. **U.S. Lead Institution:** University of Georgia (UGA)
- Principal Investigator: Dr. James W. Demski, Georgia  
Experiment Station, Experiment, GA
- Co-Principal Investigator: Dr. Cedric Kuhn, Department of Plant  
Pathology, UGA, Athens, GA
- Technician: Mr. James Chalkley, Georgia  
Exp. Station, Experiment, GA
- B. **Nigerian Counterpart Inst:** Institute for Agricultural Research (IAR) at  
Ahmadu Bello University, Samaru, PMB1044, Nigeria
- Graduate student: Mrs. Phindile Olorunju
- Nigerian advisor: Dr. Steve Misari
- C. **Thailand Counterpart Inst.** Faculty of Agriculture, Khon  
Kaen University, Khon Kaen 40002, Thailand
- Principal Investigator: Dr. Sopone Wongkaew

- D. The Philippines will probably become a cooperating institute but has not been confirmed as of September 1988. Expected linkage is:

Philippines Counterpart Inst.: Institute of Plant Breeding  
University of the Philippines at  
Los Banos, Los Banos, Philippines

Principal Investigator: Dr. Marina Natural

- C. Informal Cooperation: ICRISAT, Patancheru P.O., A.P., 502324, India

Principal Investigator: Dr. D.V.R. Reddy

## TRAINING OUTPUT

### A. Degree Training

<u>Surname</u>	<u>Sex</u>	<u>University</u>	<u>Department</u>	<u>Degree</u>	<u>Date Degree Received</u>	<u>CRSP Support</u>
<b>Nigerian Citizen:</b>						
Olorunju	F	UGA	Plant Path	PhD		Partial
<b>Thailand Citizen:</b>						
Sukornthaman	F	UGA	Plant Path	PhD	1987	Partial
<b>Others:</b>						
Warwick	F	UGA	Plant Path	PhD	1987	Partial

### B. Non-Degree Training

<u>Surname</u>	<u>Sex</u>	<u>Affiliation</u>	<u>Training</u>	<u>Location</u>	<u>Duration</u>
<b>Nigerian Citizens:</b>					
Wayo	M	IAR	Serology	Nigeria	1 week
Sule	M	IAR	Serology	Nigeria	1 week
Ansa	M	IAR	Molecular Biology	Scotland	1 month
Ansa	M	IAR	Virus Purification	Germany	1 month
Misari	M	IAR	Serology	UGA	1 week
Misari	M	IAR	Peanut Production	UGA	1 week

## ACCOMPLISHMENTS IN DETAIL

### Single and Mixed Injections with GRV and GRAV

It is widely recognized today that two viruses are associated with the groundnut rosette disease. Groundnut rosette virus (GRV) is believed to be responsible for the disease. Groundnut rosette assistor virus (GRAV) causes no overt symptoms; however, the coat protein of GRAV is believed to encapsidate the GRV-RNA, and the coat protein is essential for aphid transmission. During the past year, preliminary studies have indicated that GRAV may play a role in the severity of the rosette disease. In the first test (Table 1), mixed infections with GRV and GRAV caused a greater reduction in the length of the main stem than a single infection with GRV. In the second test (Table 2), shoot wt. but not plant heights and root wt. was reduced more by the mixed infection than by GRV alone.

Surprisingly, GRV alone did not reduce the number of pegs and pods when compared to uninoculated control plants. However, the mixed infection reduced the number of pegs and pods and pod wt. to very low values, relatively similar to production under field conditions. One implication of these preliminary results is that GRAV alone may reduce yields of peanut genotypes resistant to GRV, even though the plants appear symptomless. Additional experiments are underway.

**Table 1.** Single and mixed infections with groundnut rosette virus (GRV) and groundnut rosette assistor virus (GRAV) in peanut cultivar F 452.4

Treatment	Plant height (cm)	Dry wt/plant		No./plant		Wt. of pods (g)
		Shoots	Roots	Pegs	Pods	
Uninoculated	16.7	24.0	3.2	21	16	9.8
GRV	8.8	13.3	1.6	22	16	6.4
GRV + GRAV	9.9	7.2	4.0	7	4	0.8

**Table 2.** Single and mixed infections with the viruses causing chlorotic and green groundnut rosette in peanut cultivar MK 374

Time after inoculation (wk)	Length of main stem (cm)				Uninoculated
	Chlorotic rosette		Green rosette		
	Mech <sup>a</sup> (GRV)	Aphid <sup>b</sup> (GRV + GRAV)	Mech (GRV)	Aphid (GRV + GRAV)	
5	11.4	9.4	12.5	9.5	20.3
10	13.0	11.2	15.1	10.5	24.6

<sup>a</sup>Mechanical inoculation with groundnut rosette virus (GRV) only.

<sup>b</sup>Aphid inoculation with both GRV and groundnut rosette assistor virus (GRAV).

### Selection of peanut lines for release in Nigeria

Thirty nine groundnut lines/varieties comprising 12 early and 27 medium/late maturing lines/varieties were yield tested in 19 locations in Northern Nigeria with the view to measuring their varietal response to environments. The twelve early maturing lines planted in the Sudan savanna zone and parts of the Northern Guinea savanna zone included 4 exotic breeds, Argentine, 48-115B, 55-437 and 55-60. Similarly the 27 medium/late maturing lines which included 4 exotic breeds, RMP-12, RMP-91, 59-127 and 69-101, were tested in the Northern and Southern Guinea savanna zones.

Mean yields showed great variation with locations and years. Best yields were obtained from the Southern Guinea savanna locations where mean yields ranged from 730 to 2,933 kg/ha. In the Sudan and Northern Guinea savanna zones mean yields ranged from 385 to 1,689 kg/ha and 608 to 2,220 kg/ha, respectively.

Though observed to be susceptible to both leafspots and groundnut rosette virus (GRV), four early maturing lines (Table 3), Resistant Red Bulk (RRB), K879.78 (all IAR crossbreds), 48-115B and 55-437 (Ex-Dakar) that have exhibited good agronomic characteristics, high adaptability to the drought prone areas and high yield potential have been recommended for release for the Sudan savanna zone and parts of the Northern Guinea savanna zone. Recommended for release for the Northern and Southern Guinea savanna zones were M554-76 (IAR crossbred), RMP-12 and RMP-91, both developed in Burkina Faso (Table 4). These three have been selected based on a combination of

desirable factors of adaptability, good agronomic qualities, resistant to both strains of groundnut rosette virus (Table 5) and high yield potential.

**Table 3. Summary of mean yields, and stability measurements for early maturing varieties**

Variety	Mean* yield t/ha	Number of locations	% Yield over local	Regression coefficient (b)	S.E. (b)	Coefficient of determination (R <sup>2</sup> %)
Local	0.73b	8	-	0.72	0.062	58.0
55-437	1.20a	9	64.4	0.96	0.009	86.0
RRB	1.22a	9	67.1	0.90	0.003	80.0
K879.78	1.12a	8	53.4	1.09	0.006	98.0
48-115B	1.29a	7	76.7	0.96	0.009	93.0
Argentine	1.15a	7	57.5	0.93	0.016	92.0
55-60	1.15a	7	57.5	1.00	0.076	73.0

\*Mean yields followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

**Table 4. Summary of mean yields, and stability measurements for late maturing varieties**

Variety	Mean* yield t/ha	Number of locations	% Yield over local	Regression coefficient (b)	S.E. (b)	Coefficient of determination (R <sup>2</sup> %)
Local	1.36b	9	--	0.97	0.118	55.0
M25.68	1.67a	9	22.7	0.97	0.007	92.0
RMP-12	1.68a	10	23.5	1.02	0.002	98.0
RMP-91	1.73a	10	27.2	1.05	0.004	97.0
M554.76	1.77a	10	30.1	1.01	0.002	97.0
M1204.78 <sup>1</sup>	1.39b	8	2.2	0.72	0.009	90.0
69-101	1.37b	8	0.7	0.96	0.033	93.0

\*Mean yields followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

**Table 5. Reaction of late maturing groundnut varieties to green and chlorotic rosette strains\***

Variety	Field screening		Greenhouse** screening		Remarks
	% Green	% Chlorotic	% Green	% Chlorotic	
Sam. 38	80.00	75.00	--	--	Highly susceptible
M25.68	0.00	0.00	0.00	00.00	Resistant
RMP-12	0.00	0.00	0.00	0.00	"
RMP-91	0.00	0.00	0.00	0.00	"
M554.76	0.00	0.00	0.00	0.00	"
69-101	0.00	0.00	0.00	0.00	"
M558.79	37.7	19.10	52.60	21.10	Susceptible
M1204.78	16.00	13.00	56.50	43.50	"
M804.78	7.00	11.00	25.00	55.00	"
55-127	10.00	15.00	39.10	45.00	"
M516.79	0.00	0.00	0.00	0.00	Resistant
M585.79	0.00	0.00	0.00	0.00	"

\*Diseased plants scored as percent of total plants per plot.

\*\*Artificially inoculated with aphids.

#### Yield Loss Assessment for Peanut Stripe Virus in Groundnuts

Five groundnut cultivars (Tainan 9, Sk 38, Lampung, Mocket, and RCM 387) were assessed for yield loss due to peanut stripe virus infection under field conditions at Khon Kaen. The plants were mechanically inoculated with the ring spot variant of PStV, propagated in KC-84 R cowpea, at two weeks after emergence using a high pressure spray-gun. After three weeks, 40-50% of the plants in the inoculated plots became infected, but none was detected in the uninoculated counterparts. Natural virus spread started to occur at four weeks, and when the plants reached 80 days the numbers of infected plants in the inoculated and uninoculated plots were similar. The virus appeared to reduce pod yields of SK38, Mocket, and Lampung to a certain extent but had no effect on Tainan 9 and RCM 387 except it increased the number of shrivelled seeds in Tainan 9 considerably. Among the five cultivars tested, RCM 387 showed the highest level of tolerance to PStV infection as its seed quality and pod yield were not affected (Table 6).

**Table 6. Effect of the peanut stripe virus ringspot variant on yield of five groundnut cultivars grown under field condition at Khon Kaen**

Cultivar <sup>1</sup>	Treatment	Yield component <sup>2</sup>			
		Pod dry wt. (g/10 plants)	Yield reduction (%)	Shelling (%)	Shriveled seed (%)
Sk 38	Control	59.3		29.0	24.8
	Inoculated	47.8	19.4	25.5	19.8
	F test (p = 0.05)	*		NS	NS
	C.V. (%)	6.3		13.8	46.2
Tainan 9	Control	73.3		23.3	15.3
	Inoculated	72.3	1.4	25.5	33.5
	F test (p = 0.05)	72.3		NS	*
	C.V. (%)	26.7		10.2	19.8
Lampang	Control	89.0		27.8	18.0
	Inoculated	67.5	24.2	26.0	22.0
	F test	NS		NS	NS
	C.V. (%)	10.0		14.9	18.22
Moket	Control	87.5		28.3	19.5
	Inoculated	69.0	21.1	27.0	21.5
	F test	NS		NS	NS
	C.V. (%)	12.6		8.4	14.1
RCM 387	Control	72.5		32.3	28.0
	Inoculated	77.5	+5.0	30.0	29.0
	F test	NS		NS	NS
	C.V. (%)	19.45		11.3	17.4

<sup>1</sup> Statistical analyses were done separately for each groundnut cultivar.

<sup>2</sup> Averaged from 4 replicates of 10 infected plants in the inoculated plots and 10 healthy plants in the control plots.

### Purification and Serology of Peanut Stripe Virus

The experiment was conducted to compare the efficacy of procedures currently employed for potyvirus purification and to investigate the serological relationship among PStV symptom variants and other legume potyviruses. By using the ringspot variant (PStV-R) propagated in cowpea (*Vigna unguiculata* KC-84) as a virus source and the purification procedures as described by Fukomoto *et al.* (1986), no virus band could be detected in the sucrose gradient. The combined procedure of that of Lima *et al.* (1979) and Demski and Reddy (1987) yielded 4-5 mg of purified virus per 100 g of leaf tissue. The particles appeared intact under the electron microscope and were infectious. The same procedure when applied to the green blotch variant (PStV-G) yielded no detectable virus particles. The opalescent band formed 1.5 cm from the meniscus of cesium chloride gradients contained only a polyribosome-like material. The antiserum prepared from the purified PStV-R reacted strongly with the purified preparation and with extracts taken from PStV-R and PStV-M (mild mottle variant) infected leaves treated with 1.5% SDS. The test was done in an agar gel double diffusion system as described by Purcifull and Batchelor (1977). Extracts from blackeye cowpea mosaic virus (BICMV) and the PStV-G infected leaves reacted only weakly to the PStV-R antiserum. Extracts from both soybean mosaic virus (SMV) infected leaves and healthy plants gave a negative result. By using a reciprocal test in which antisera of peanut chlorotic ring mottle (PCRMV), SMV, BICMV and peanut mottle virus (PMV) were used as references, all PStV variants appeared to react strongly with the antisera of PCRMV and BICMV. A mild reaction with spur formation was observed between the

wells that contained purified preparation of virus indicating a distant relationship between the two viruses. No relationship was observed among these viruses and PMV.

#### Procedure for purification of tomato spotted wilt virus (TSWV)

1. Harvest the systemically infected *Nicotiana tabacum* cv. Burley 21 leaves, derib the leaves, and weigh them.
2. Grind the leaves in Waring blender using 0.1 M KPO<sub>4</sub> buffer, PH 7.0 + 0.01 M Na<sub>2</sub>SO<sub>3</sub> (0.01 M sodium diethyldithiocarbamate may be used) at 1 g : 3 ml ratio. Use precooled buffer and blender in cold room (4° C). Perform all purification steps in cold room.
3. Squeeze the extract through four layers of cheese cloth and discard the debris in the cloth.
4. Centrifuge the extract: 4500 rpm, 15 min, 4°C.
5. Save the supernatant and record its volume.
6. Add PEG (6000 Mol. Wt., Fisher Product) to 4% and NaCl to 0.2 M to the supernatant while stirring in cold. Dissolve the chemicals and leave the preparation in cold for 90 minutes to 2 hours.
7. Centrifuge: 9,900 g, 20 minutes, 4°C. Discard the supernatant.
8. Resuspend the pellets in cold 0.1 M KPO<sub>4</sub>, pH 7.0 buffer containing 0.01 M Na<sub>2</sub>SO<sub>3</sub>. Use minimal volume depending on the number sucrose gradient tubes to be used in Step 10.
9. Centrifuge: 9000 g, 20 minutes, 4°C and save the supernatant.
10. Layer the supernatant on preformed 10-40% (8 ml each concentration) linear sucrose gradients prepared in 0.1 M KPO<sub>4</sub>, pH 7.0 + 0.01 M Na<sub>2</sub>SO<sub>3</sub> and centrifuge: 25,000 rpm, 1 hr, 5°C (SW 28 rotor).
11. Observe the tubes in dark room for light-scattering zones (see Figure).
12. Collect the zones separately (use a syringe with a bent needle), dilute with 0.1 M KPO<sub>4</sub>, pH 7.0 + 0.01 M Na<sub>2</sub>SO<sub>3</sub> and pellet the virus: 35,000 rpm, 1 hr, 5°C. or Layer the diluted virus zones (without pelleting) directly onto 25-50% preformed linear sucrose gradients (8 ml each concentration) in 0.1 M KPO<sub>4</sub> buffer, PH 7.0 + 0.01 M Na<sub>2</sub>SO<sub>3</sub>
13. Resuspend the pellets of step 12 in 0.1 M KPO<sub>4</sub>, pH 7.0 + 0.01 M Na<sub>2</sub>SO<sub>3</sub> (minimal volume) and layer on 25-50% sucrose gradients as mentioned in the previous step. Centrifuge: 25,000 rpm, 6 hr, 5°C (SW 28 rotor).
14. Follow steps 11 and 12, pellet the virus, and resuspend it in 0.1 M KPO<sub>4</sub> buffer, pH 7.0 +0.01 M Na<sub>2</sub>SO<sub>3</sub>.
15. If still green, process the preparation through one more 25-50% sucrose gradient.
16. Use the final preparation for infectivity assay, serology, electron microscopy and for biochemical studies.

From the purified TSWV, an antiserum was produced and serological comparison to other TSWV isolates was completed. Little differences were detected among isolates of TSWV obtained from different hosts in the U. S. However, the U. S. isolate of TSWV from peanut is serologically different from an isolate of TSWV from peanut in India.

The virus proteins from Texas a isolate were resolved into five polypeptides on sodium dodecyl sulphate-polyacrylamide slab gels, and their molecular weights ranged from 64 x 10<sup>3</sup> to 26 x 10<sup>3</sup> daltons. In the electro-blot immunoanalysis all five virus polypeptides of the Texas isolate reacted with its homologous, North Carolina and Australian isolates antisera, but only the two highest molecular weight polypeptides reacted with the Indian isolate antiserum, indicating that the Texas isolate is serologically distinct from the Indian isolate.

#### Comparison of five ELISA procedures for detection of peanut stripe virus

Protein A sandwich (PAS) and plate-trapped antigen (PTA) forms of indirect enzyme-linked immunosorbent assay (ELISA), and standard double-antibody sandwich (SDAS), two-step modified DAS (MDAS), and simplified rapid direct antigen coating (SRDAC) forms of direct ELISA were compared for the detection of peanut stripe virus (PSV) under short incubation periods (90 min) at 37°C. The virus was detectable in peanut seeds and white lupine leaves at 1:10,000 dilutions of wheat by all five procedures. But in peanut leaves it was detectable at 1:1,000 dilution by SDAS, SRDAS, PAS and PTA and not by MDAS. The absorbance readings were higher for PTA and SRDAC forms and were least for MDAS form (Table 7). Of the two enzyme conjugates used in the

direct ELISA, penicillinase system detected the virus in peanut leaves at 1:10000 dilutions that were negative with alkaline phosphatase system. The sensitivity of virus detection for SRDAC, PAS and PTA procedures was comparable to SDAS with respect to peanut seed and white lupine leaf virus source. But the sensitivity of SRDAC and PTA methods using peanut leaves as a virus source, was higher compared to three other forms of ELISA.

**Table 7. Detection of peanut stripe virus (PStV) in peanut leaves and seeds and white lupine leaves by standard double antibody sandwich (SDAS), two step modified DAS (MDAS) and simplified rapid direct antigen coating (SRDAC) forms of direct ELISA based on alkaline phosphatase**

Antigen sample	SDAS		MDAS		SRDAC		
	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	
Peanut leaves							
Healthy 1:100	0.00 <sup>a</sup>	0.00-0.00	0.02	0.00-0.04	0.03	0.02-0.03	
	0.00	0.00-0.00	0.02	0.00-0.05	0.03	0.02-0.05	
Infected 1:100	0.26	0.16-0.37	0.10	0.08-0.13	1.29	0.99-1.53	
	0.18	0.10-0.27	0.10	0.08-0.11	0.99	0.70-1.26	
	0.19	0.09-0.29	0.10	0.05-0.15	0.37	0.32-0.48	
Peanut seeds							
Healthy 1:100	0.03	0.02-0.05	0.03	0.02-0.07	0.02	0.00-0.04	
	0.02	0.01-0.02	0.02	0.05-0.01	0.01	0.00-0.03	
Infected 1:100	1.59	1.12-2 <sup>b</sup>	0.85	0.52-1.18	1.69	1.33-2+	
	1.49	0.96-2+	0.61	0.34-0.89	1.26	0.87-1.66	
	0.48	0.32-0.65	0.17	0.15-0.20	0.29	0.20-0.44	
White lupine leaves							
Healthy 1:100	0.03	0.02-0.04	0.02	0.00-0.04	0.01	0.00-0.02	
	0.01	0.00-0.04	0.01	0.00-0.03	0.03	0.00-0.03	
Infected 1:100	1.83	1.64-2+	0.73	0.32-1.15	1.65	1.22-2+	
	1.40	0.78-2+	0.35	0.22-0.55	1.60	1.18-2+	
	1.46	0.90-2+	0.37	0.17-0.56	1.44	1.06-1.82	

<sup>a</sup> Absorbance ( $A_{410}$ ) values represent average of three experiments.

<sup>b</sup> Readings over 2 were recorded as 2+, and for calculation of  $\bar{X}$  these values were considered as 2.00.



### Serology tests for luteovirus in peanut in Georgia

In 1987 leaf samples were collected from 20 commercial peanut fields in Georgia. A total of 780 plants were tested by enzyme-linked immunosorbent assays (ELISA) with antisera to two luteoviruses, potato leafroll virus (PLRV) and beet western yellows virus (BWYV). Positive ELISA absorbance readings were found for 44 and 37 plants with PLRV and BWYV antisera, respectively. The standards for positive readings were a minimum absorbance of 0.1 and absorbance of at least two-fold greater than controls (peanut plants grown in a greenhouse, protected from aphids). Furthermore, similar results were found with peanut plants grown in experimental plots near Athens, Georgia. When 280 plants were tested by ELISA, 10 and 12 plants were positive with PLRV and BWYV antisera, respectively. Since definitive transmission tests have not been conducted, no conclusions about the presence of luteovirus in peanuts in Georgia can be made at this time. However, the serology results warrant future critical studies.

### Application from research

The release of 20 tons of the groundnut rosette resistant line RMP-12 in 1988 for use by peanut growers is a start aimed at reversing the downward trend of peanut production in Nigeria and West Africa. Although RMP-12 is a 120 day peanut line, it can be grown in areas south of Zaria, Nigeria, where rainfall is sufficient to grow a long season crop. Other peanut lines have been released to the Nigerian Seed Development Commission and increase in 1988 should permit multiple line distribution in 1989. In areas north of Zaria the growers should use the IPM program that was developed at the Institute for Agricultural Research until resistant lines that mature in less than 100 days are developed.

### RESEARCH PLAN

1988 will be a year of transition with the phase down in Nigeria and the buildup in S. E. Asia. The Nigerian graduate student (Phindile Olorunju) will be supported until she graduates which is expected to be December 1989.

1988 - 1990 emphasis is being placed on the development of a PStV virus-free seed program in S. E. Asia. Seed of selected peanut lines will be produced in a greenhouse from only healthy plants. These seed will then be field increased in areas that have been selected as not having PStV. Seed from this increase will be used in areas where PStV has been endemic to determine if virus-free seed are economically feasible to reduce the incidence of the virus diseases. Dr. Sopone Wongkaew will work in the virus lab at Montpellier, France for several months to compare the numerous isolates of PStV under similar conditions (France will permit the importation of the isolates from different world areas). A continued search for peanut lines that are resistant to PStV will be done.

### Publications

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## Influence of Soil Microbiology on Nitrogen Fixation and Growth of Peanuts in Thailand and Philippines

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### INTRODUCTION

Past CRSP-supported research at NCSU in biological nitrogen fixation (BNF) in peanut has accomplished the following pertinent to the current annual report:

1. We have shown that BNF, as a process, is equally controlled by the plant host (peanut) and the bacterial symbiont (*Bradyrhizobium* sp.),
2. Symbiotic nitrogen fixation in peanut can be improved by genetically manipulating the host plant,
3. Symbiotic nitrogen fixation in peanut can also be improved by selection and genetic manipulation of the bacterial symbiont, and
4. To obtain optimum BNF, the cultivar and bacterium must be "matched."

The major accomplishments in the host countries also resulted from the above findings. Manipulation of the cultivar and *Rhizobium* resulted in significant yield increases in some countries. One of the CRSP-supported areas was in Cameroon, Africa. In North Cameroon, peanuts are the primary cultivated legume, covering 120,000 ha. Cultivar 28-206 accounts for 90% of this acreage, and this cultivar has given 28% yield increases in the field when inoculated with NC92, a *Rhizobium* introduced through this program. This translates into an average yield response of 182 kg/ha, or a total of 20,000 MT/season. It should be noted that all of these peanuts are locally consumed as food. A Cameroonian company is now producing the appropriate inoculum. A recent letter to the P.I. (July 13, 1988) from David Bathrick, Director of the Office of Agriculture, Bureau for Science and Technology, USAID states that at a world price average of \$400/MT, this technology, if adopted fully by Cameroon, is worth U.S. \$8,000,000 to that country's economy. In Thailand and the Philippines, results were not as consistent as in Cameroon until recently.

Field studies at Khon Kaen University (Dr. Banyong Toomsan) have shown significant yield increases in peanuts inoculated with *Rhizobium* strains selected through the NCSU project. We are excited about these results since previously we could not measure consistent beneficial effects in yield due to BNF. The limiting factor has been identified as a deficiency of trace elements, especially molybdenum, in northeast Thailand. When the soils were supplemented with Mo, BNF increased and significant yield increases resulted. Since yields are still lower than optimum, other limiting factors will be determined, pending availability of funds.

### GOALS AND OBJECTIVES

The long-term and continuing goal is to optimize biological nitrogen fixation to increase peanut yields in the HC, and as part of a farming systems approach, use BNF as a source of nitrogen fertilizer for crops grown in rotation or intercropped. To do this, the constraints to BNF in the host plant and/or the nitrogen-fixing bacteria must be determined. This requires a joint effort between plant breeders and microbiologists.

## ORGANIZATION AND APPROACH

Due to budget cuts we received no operating money during FY 88 for the domestic program. All money received was used to pay precommitted salaries. No money was recommended for the overseas collaborators. In spite of this, both the domestic research, as well as a modest research program in Thailand and the Philippines, continued. Temporary domestic operating funds were obtained from the N. C. Agricultural Research Service to allow continuation. This has required a change in organizational structure. A technician position, previously CRSP-funded, has been eliminated. Four students (see TRAINING OUTPUTS) doing thesis work on peanut CRSP projects have graduated with the PhD degree (three of these were on CRSP-funded stipends). One M.S. candidate (not on CRSP stipend) has completed CRSP-supported research. There are two continuing Ph.D.-level students, both on CRSP stipends. Two new students (one Ph.D. level and one M.S. candidate) are continuing CRSP research but are on university-funded graduate assistantships. Thus, graduate student participation has decreased from seven (5 on CRSP stipends) to four (2 on CRSP stipends).

Our approach continues to be a cooperative one with the peanut breeding component (Dr. J. C. Wynne). Cultivar breeding is central to the CRSP objectives and we see our approach as a mutualistic part of this plant breeding program. Biological nitrogen fixation, pest and disease resistance, etc. are all inputs required by the plant breeder to optimize peanut yield in the host countries.

The following collaborators of record remain, although because of budget cuts not all of them were active:

- A. U.S. Lead Institution:** North Carolina State University, Raleigh  
 Principal Investigator: Dr. G. H. Elkan, Dept. of Microbiology  
 Cooperator: Dr. J. C. Wynne, Dept. of Crop Science  
 Institutional Representative: Dr. B. E. Caldwell, Head, Dept. of Crop Science
- B. S.E. Asian Counterpart Institutions:** Department of Agriculture  
 (DOA) and Khon Kaen University (KKU), Thailand; University of the Philippines at Los Banos  
 (UPLB), Philippines  
 Project Coordinators: Dr. Erlinda Paterno, UPLB, Philippines  
 Dr. Vichitr Benjasil, DOA, Thailand  
 Principal Investigators: Dr. Erlinda Paterno, UPLB, Philippines  
 Dr. Nantakorn Boonkerd, DOA, Thailand  
 Co-Principal Investigators: Dr. Banyong Toomsan, KKU, Thailand  
 Cooperators: Mrs. Fe G. Torres, UPLB, Philippines  
 Dr. Teresita Espino, UPLB, Philippines  
 Dr. Randy A. Hautea, UPLB, Philippines  
 Mr. Billy Temanuel, Isabela State Univ., Philippines  
 Mrs. Yenchai Vasuvat, DOA, Thailand  
 Dr. Montien Somabhi, KKU, Thailand  
 Dr. Aran Patanothai, KKU, Thailand
- C. USAID Project Officers:** Mr. Robert Resseguie, USAID/Manila  
 Mr. Douglas Clark, USAID/Bangkok

## ACCOMPLISHMENTS

As a result of the findings listed above, a number of studies were initiated. The purpose of these is to answer the questions resulting from these:

1. If BNF can be improved by cultivar selection and breeding, what is being enhanced or changed by this technology (*i.e.*, is there more energy made available to the bacteria in the nodules? If so, in what form? Or, is transport of the fixed nitrogen improved and, if so, in what form?)

2. How genetically stable are these bacteria after going through a symbiotic cycle through an altered host (inoculum is usually applied fresh each season, what happens to the BNF properties of the bacteria when released from nodules, then infected subsequent plantings)?

Approaching the first question involves examining back crossed cultivars, both uninoculated and inoculated with single strain mutant bacteria in order to measure the transport amino acids, free amino acids and free sugars (energy sources) in both nodule and peanut fruit. The bacteria are capable of invading the roots of legumes, inducing the development of nodules and differentiating into bacteroids which convert dinitrogen into ammonia. The host plant provides the carbon and energy sources for the bacteroids while assimilating and exporting the ammonia produced in the nodule.

It is generally considered that the plant regulates the energy supplied to the bacteroids and the form in which newly fixed nitrogen is translocated from nodules to the shoot system. The bacteroids provide the  $N_2$ -fixing system and the heme moiety of the oxygen-binding molecule leghemoglobin but are not thought to play a role in the regulation of nitrogen metabolism in the plant.

Our basic premise is that bradyrhizobia are involved in more than just fixing  $N_2$  in order to supply the host plant with a reduced form of nitrogen. The effects of interactions between bacterial strain and host plant on efficiency of nitrogen fixation, plant weight, pod yield, total plant nitrogen and nodule number are well documented. However, in spite of evidence suggesting that *Bradyrhizobium* strains can affect nodule amino acid composition and nitrogen translocation, little has been done to determine the extent of influence that particular strains have on nodule and seed composition in the peanut symbiosis.

The specific objective of this study was to determine the effects of *Bradyrhizobium* sp. strains on (1) the free amino acid composition of peanut (*Arachis hypogaea*) root nodules and (2) the fatty acid, free sugar and free amino acid content of the peanut seed.

The plant material used in this study resulted from determination of the inheritance of several  $N_2$  fixation-related traits for two peanut crosses, to test Mather and Jink's additive-dominance model, and to determine if epistasis was important in the inheritance of these traits. A generation means analysis using parents, reciprocal  $F_1$ s and  $F_2$ s and the two backcross generations was conducted for both crosses. Plants of different generations were greenhouse-grown in modified Leonard jars for about 60 days at which time nodule number and dry weight, shoot dry weight, nitrogenase activity and specific activity were measured. Means of the traits for the generations from both crosses (Robert 33-1 x NC 4 and Robert 33-1 x Argentine) showed significant differences. Reciprocal differences were found for most traits measured in the cross of Robert 33-1 x Argentine, a cross of Virginia x Spanish botanical types. Lack of fit of the additive-dominance model indicated significant epistasis for inheritance of nodule number, nodule weight, top dry weight, and nitrogenase activity in two crosses. Three types of digenic interactions (additive x additive, additive x dominance and dominance x dominance) were found. The presence of nonadditive genetic effects suggests that early generation selection would be ineffective. These plants were then used in greenhouse and field studies.

Nitrogen fixation in legumes requires absolute cooperation between symbiotic partners. Consequently, it is important to understand the contributions made to the association by each partner, particularly for those reactions brought about through a concerted effort of host and strain. Most studies on the effects of host-strain interactions in the *Bradyrhizobium*-peanut symbiosis have been directed toward understanding the relationships between nodulation, efficiency of nitrogen fixation and seed yield. Little is known of the effects of these interactions on the types of nitrogen-transporting compounds produced in the nodule from bacteroid-generated ammonia, or of the fate of newly assimilated nitrogen translocated from nodules into root xylem sap. Furthermore, the role of the bacteroid in the regulation of nitrogen metabolism in the nodule generally has been perceived to be a passive one. The host is considered to be in control of ammonia assimilation and synthesis of nitrogenous transport compounds. The influence of *Bradyrhizobium* sp. on the types and concentrations of nitrogen-transporting compounds produced in the nodule has not been studied in depth.

The first stable product of  $N_2$  fixation in legume nodules is ammonia which is readily incorporated into glutamine and glutamic acid within the nodule. These amino acids are subsequently converted

into one or more nitrogen-transporting compounds depending, in part, on the species of host plant. In the case of *Arachis*, there has been controversy as to the form of transported nitrogen with asparagine or 4-methylene glutamine being the prime candidates.

The influence of *Bradyrhizobium* sp. strains on the fatty acid, free sugar and free amino acid content of *A. hypogaea* was investigated in greenhouse and field experiments. Free amino acids were analyzed as phenylthiocarbamyl derivatives by reverse-phase high-performance liquid chromatography. Free sugars and lipid-derived fatty acids were methylated and measured by gas chromatography.

The effect of inoculum on the amino acid composition of peanut root nodules was determined in a greenhouse experiment in which Spanish- and Virginia-type cultivars were inoculated with various *Bradyrhizobium* strains and grown to maturity. Nodules collected at regular intervals during plant growth showed strain-dependent variations in levels of asparagine, aspartic acid, 4-methyl-eneglutamine, proline and an unknown amino acid.

The influence of strain on lipid fatty acids, free sugars and free amino acids in seed was evaluated in field-grown peanuts using Spanish- and Virginia-type cultivars. Uninoculated (control) plants showed significant differences in seed levels of stearic acid, arachidic acid, glucose, raffinose and stachyose compared to effectively nodulated peanut. Strain-dependent variations in seed free amino acid content were significant for asparagine, histidine, arginine, methionine, phenylalanine and serine.

These data suggest that the types and concentrations of free amino acids produced in the seeds and root nodules of peanut are, in part, dependent on the *Bradyrhizobium* strain used for inoculation.

### Seed Fatty Acid Composition

Variation in the fatty acid content of peanut seed oil from different cultivar-strain combinations was found. Palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2) were the major fatty acids in all samples, ranging from 8.1-11.9%, 37.6-60.9% and 21.3-41.1%, respectively. Other fatty acids present in all samples were stearic (18:0), arachidic (20:0), eicosanoic (20:1), behenic (22:0) and lignoceric (24:0). Oleic/linoleic ratios ranged from 0.915-2.883. These two fatty acids comprised 76.1-82.2% of the total. O/L ratios were highest for cultivar NC 7 (mean = 2.550) and lowest for Robut 33-1 (mean = 0.945), indicating that oil from NC 7 would be least prone to oxidation.

Table 1 shows the results of analysis of variance in seed fatty acid composition. Cultivar effects were significant at the 0.1% level for all fatty acids, the O/L ratio and the seed-hull maturity index. Similar results for variety effects have been reported in several previous studies. A treatment effect at the 0.1% level of significance and a cultivar-treatment interaction at the 5% significance level were found for stearic and arachidic acids. These effects were associated with uninoculated plants, especially cultivars NC 7 and Argentine, which showed significantly higher levels of stearic and arachidic acids than other treatments. There were no significant variations in seed fatty acid content as a result of inoculum strain.

### Seed Sugar Content

Variation in the free sugar composition of peanut seed from different cultivar-strain combinations were found. All samples contained detectable levels of seven sugars. The major sugar in all samples was sucrose (83.7% of the total), ranging in concentration from 17.0-38.1 mg/g fat-free meal. Other sugars present were (in order of descending concentration) raffinose, stachyose, fructose, glucose, inositol and ribose. Seed from Argentine had considerably less sucrose and total sugar than Virginia-type peanuts. The mean values of sucrose for Florigiant, Robut 33-1, NC 7 and Argentine were 28.6, 28.2, 24.3 and 20.0 mg/g, respectively. Raffinose and stachyose were the only sugars found at >1 mg/g defatted seed. These sugars comprised 6.6 and 5.9% of the sugar total. The sucrose content of Virginia-type peanuts from this study is similar to that reported previously, but the concentrations of other sugars are different. The mean concentration of stachyose was approximately half of that reported by Oupadissakoon in Thailand, while raffinose, fructose and inositol values were much higher. Oupadissakoon found seed sucrose levels of 27.0-37.5 mg/g fat-free meal and means for stachyose, raffinose, fructose and inositol of 3.98, 0.53, 0.21 and 0.13 mg/g defatted seed,



respectively. Discrepancies between the present study and previous investigations may be the result of differences in cultivars and sugar derivitization methods used.

Table 1. Analysis of variance in fatty acid composition of peanuts from four cultivars inoculated with various *Bradyrhizobium* strains<sup>a</sup>

	-----Analysis of variance with mean squares-----				
	Clv	Trt	Clv x Trt	GM	Error
df	3	4	12	--	20
16:0	17.912***	0.161	0.280	10.526	0.164
18:0	7.854***	1.579***	0.267*	2.897	0.099
18:1	766.947***	4.243	1.953	47.127	1.697
18:2	535.653***	0.540	1.954	32.694	1.126
20:0	0.586***	0.123***	0.023*	1.439	0.009
20:1	0.727***	0.035	0.009	9.592	0.013
22:0	1.549***	0.035	0.077	2.828	0.052
24:0	0.632***	0.013	0.024	1.312	0.014
O/L	4.914***	0.021	0.027	1.561	0.017
SHMI	2.566***	0.046	0.109	2.990	0.142

Clv = cultivar, Trt = treatment, GM = grand mean, df = degrees of freedom, O/L = oleic/linoleic ratio and SHMI = seed/hull maturity index. \*,\*\*\*Mean square is significant at 5 and 0.1% level of probability, respectively.

The results of analysis of variance in seed sugar composition are shown in Table 2. Variations due to cultivar were significant at the 0.1% level for fructose, glucose, sucrose, raffinose, stachyose and SHMI. Glucose, raffinose and stachyose varied with treatment at the 5, 1 and 0.1% levels of significance, respectively. A cultivar-treatment effect for stachyose was significant at the 0.1% level. Uninoculated control plants were the source of variation in raffinose and stachyose levels. Seed from this treatment had significantly higher concentrations of raffinose and stachyose than those from inoculated plants. Seed from plants inoculated with RP182-13 or 176A22 showed significantly lower seed glucose levels when compared with uninoculated controls. All other differences were nonsignificant.

**Table 2. Analysis of variance in free sugar content of seed from four peanut cultivars inoculated with various *Bradyrhizobium* strains<sup>a</sup>**

	-----Analysis of variance with mean squares-----				
	Clv	Trt	Clv x Trt	GM	Error
df	3	4	12	--	20
Ribose	0.032	0.006	0.006	0.034	0.017
Inositol	0.111	0.083	0.092	0.252	0.117
Fructose	7.017***	0.698	0.716	0.504	0.411
Glucose	2.112***	0.355*	0.173	0.373	0.108
Sucrose	5423.137***	833.365	965.066	25.275	459.823
Raffinose	79.246***	26.568**	7.126	2.007	6.025
Stachyose	139.358***	79.610***	25.094***	1.768	5.085
SHMI	2.568***	0.048	0.110	2.990	0.141

<sup>a</sup>Clv = cultivar, Trt = treatment, GM = grand mean, df = degrees of freedom and SHMI = seed/hull maturity index. \*, \*\*, \*\*\* Mean square is significant at 5, 1 and 0.1% levels of probability, respectively.

#### Free Amino Acid Composition of Seed

Cystine was present in all derivitized extracts but only in amounts too small to be accurately integrated (<1 pmol/10 microliters). The major free amino acids of peanut seed were (in order of descending concentration) alanine, phenylalanine, arginine and glutamic acid, which comprised approximately 39% of the total pool. Depending on the cultivar, the levels of these amino acids ranged from 11.6-13.4% (alanine), 9.0-14.7% (phenylalanine), 5.8- 9.9% (arginine) and 6.8-8.1% (glutamic acid).

Variations in analysis of variance due to cultivar were significant at the 0.1% level for tyrosine; at the 1% level for histidine, arginine, phenylalanine and lysine; and at the 5% level for asparagine, serine and glycine. A treatment effect on the mean values of asparagine was significant at the 0.1% level of probability. Plants inoculated with NC92 had seed asparagine levels significantly lower than plants inoculated with other bradyrhizobia. Flo 1A- treated plants showed increased levels of asparagine over plants inoculated with 176A22 or NC92. The histidine content of seed was also higher in plants inoculated with Flo 1A when compared to inoculation with RP 182-13 or to uninoculated controls. Seed from uninoculated control plants were lower in arginine and methionine content than plants inoculated with Flo 1A or RP 182- 3 and lower in methionine than plants inoculated with 176A22. Uninoculated controls and plants inoculated with NC92 had significantly lower values for serine than plants inoculated with 176A22 or Flo 1A. A significantly higher level of phenylalanine was present in seed from Flo 1A-treated plants than in seed from uninoculated controls. All other variations in seed amino acid composition were nonsignificant.

The results of statistical analyses indicate that the *Bradyrhizobium* strains used in this study do not significantly affect the lipid fatty acid or free sugar content of peanuts. Variations in fatty acids and sugars occurred only in uninoculated plants and involved only minor seed components. The major seed fatty acids (oleic, linoleic, palmitic) and the principal seed sugar (sucrose) were unaffected.

Analysis of variance in mean levels of seed amino acids suggests that certain amino acids show strain-dependent variations. In particular, the asparagine content of peanuts was influenced by *Bradyrhizobium* sp. Asparagine is thought to be the carrier of newly fixed nitrogen translocated from root nodule of 'temperate' legumes. It is possible that strains which do not fix nitrogen as effectively as others would limit the plant's supply of ammonia and directly influence seed asparagine content. Data from the present study show uninoculated plants with the lowest asparagine concentrations. Plants inoculated with NC92, a moderately effective strain, show seed asparagine levels higher than

uninoculated controls, but significantly lower than seed from plants inoculated with the more effective strains, Flo 1A or RP 182-13.

Seed from plants inoculated with different bradyrhizobia also show strain-dependent variations in seed amino acids associated with 'typical' and 'atypical' roasted peanut flavor. Flo 1A-treated plants had consistently higher levels of asparagine, histidine and phenylalanine than other treatments. These amino acids are linked to 'typical' roasted peanut flavor. Seed from uninoculated plants contained the lowest mean concentration of arginine, which is associated with 'atypical' roasted peanut flavor.

The influence of *Bradyrhizobium* sp. on the free amino acid content of root nodules from *A. hypogaea* was evaluated in two Spanish-type cultivars (Pronto and Arginine) and two Virginia-type cultivars (Robut 33-1 and NC 7) grown to maturity under controlled greenhouse conditions. Three effective strains (NC70.1, 3G4b20, NC92) and three ineffective strains (NC1.3, NC22.4, NC120) of *Bradyrhizobium* sp. were used as inocula. Free amino acids were analyzed by reverse-phase high-performance liquid chromatography of derivitized extracts from nodules collected at regular intervals during plant growth. Aspartic acid, glutamic acid, asparagine and alanine accounted for approximately 90% of the free amino acids in nodules from all cultivars inoculated with effective strains. Asparagine was the primary nodule amino acid when NC70.1 or NC92 was used, whereas aspartic acid was the major amino acid when 3G4b20 was the inoculum. In nodules from plants inoculated with ineffective strains, 4-methyleneglutamine, proline, aspartic acid, glutamic acid, asparagine and an unknown amino acid were the major nodule amino acids. When NC22.4 or NC120 were used, 4-methyleneglutamine was dominant, but proline or the unknown amino acid occurred in higher concentrations when NC1.3 was the inoculum. These data suggest that nodule amino acid composition can be altered by the activity of *Bradyrhizobium* sp.

#### "Artificial Nodule" Studies

Another approach toward understanding the regulation by the host plant of nitrogen fixation in the nodule was taken with the development in our laboratory of an artificial nodular environment. Using a carefully controlled chemostat, we are able to supply the organic components, to the rhizobia therein shown in the above summarized study, in an atmosphere simulating the nodule environment. Because of the huge energy expenditure required for nitrogen fixation, current research efforts have attempted to address possible areas where efficiency can be increased. To do this, it would be desirable to study the development and regulation that occurs within the nodule. Unfortunately, one of the major obstacles to this goal is that the metabolism and regulation of the plant and bacteroid are difficult to separate. Therefore, a long-term objective is to understand how *Bradyrhizobium* sp. (*Arachis*) responds when put under defined conditions that are similar to the nodule environment which results in the derepression of nitrogenase and if the cells from such growth conditions are similar to differentiated bacteroids. Using this strategy it is possible to understand many of the key points in the regulation and how a shift in specific environmental stimuli can affect this.

To begin to understand how this might happen, it is useful to compare the known differences that result in key metabolic pathways when a bacterium differentiates into an active nodule bacterium. In general, the nondifferentiated *Rhizobium* can use a diverse number of carbohydrates, organic acids and even amino acids as carbon and energy sources. Nitrogen is obtained from either ammonia or amino acids. In the nodule, on the other hand, bacteroids are more specialized. Recent experimental evidence suggests that nodular forms use organic acids and possibly amino acids as carbon and energy source; although the use of amino acids is presently hypothetical. In the nodule, rhizobia do not appear to directly assimilate ammonia and thus we are examining the nitrogen form used which depends on a carbon skeleton from the plant host which is excreted into the plant cytosol. Plant enzymes then use the ammonia to form amino acids which can be used as a nitrogen source for both the bacteroids and the plant.

*Ex planta* nitrogen fixation by rhizobia requires highly defined conditions. It is generally accepted that for maximum nitrogenase activity the medium should contain two carbon and energy sources and an amino acid nitrogen source. To our knowledge, the highest nitrogenase activity that has been reported for *Bradyrhizobium* sp. is 776 nmol min<sup>-1</sup>mg dry weight<sup>-1</sup> for peanut strain CB756 which was grown in a continuous culture containing 43 mM glycerol, 5 mM sodium succinate, and 2 mM glutamine. Oxygen, however, was the major variable in these experiments. When dissolved oxygen was

increased above 2.8 micromoles, nitrogenase activity was lost. This is an important difference between free-living rhizobia and bacteria in the nodule. Free-living rhizobia live in aerated conditions where nitrogen fixation is repressed while bacteroids live in microaerated conditions where nitrogen fixation is derepressed. Bacteroid metabolism is also most efficient when oxygen is delivered by oxygen-carrier molecules such as leghemoglobin. The culture used, *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20, has been shown to be extremely effective on several *A. hypogaea* cultivars and can fix nitrogen *ex planta*. Two experimental approaches were used; semisolid and broth media were used to simulate microaerobic and aerobic conditions during growth on succinate. This enabled us to examine regulatory control that oxygen and succinate have on strain 3G4b20 during growth on amino acids. Then, a succinate-limited chemostat was used to examine how the metabolism and polypeptide patterns changed during the shift from aerated to microaerated, nitrogen-fixing conditions. This enabled us to examine the changes that result in a *Bradyrhizobium* sp. (*Arachis*) culture that result from specific environmental stimuli. The data gathered from these experiments are being used as a baseline for future genetic and physiology experiments and eventually may help to understand how symbiotic nitrogen fixation is regulated.

Semisolid and broth media were used to simulate microaerobic and aerobic conditions for *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20 during growth on succinate. Succinate concentrations greater than 25 mM were inhibitory under both conditions. Growth patterns in the semisolid medium suggest that succinate inhibition may be related to an increase in oxygen sensitivity. Succinate concentrations of 35 mM resulted in swollen, pleomorphic cells that had greatly decreased viability (culturability) when grown in aerobic broth and had high nitrogenase activity when grown in semisolid media. *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20 also grew on aspartate, glutamate, asparagine, proline and alanine as sole carbon, energy and nitrogen sources; but, when 15 mM succinate was included in the medium, the growth on amino acids was totally inhibited. When the same experiment was repeated in semisolid medium, *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20 grew on every amino acid tested and had nitrogenase activity with glutamate, asparagine, histidine and aspartate. Our data are consistent with the hypothesis that succinate and oxygen act in concert to trigger morphological and physiological changes in *Bradyrhizobium* sp. (*Arachis*) that are frequently associated with bacteroid development.

The regulatory control of oxygen and succinate concentrations on growth, respiration and polypeptide patterns of *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20 was examined. A succinate-limited chemostat enabled us to vary oxygen concentrations while keeping other parameters constant. Molar growth yields ( $Y_{succ}$ ) ranged from 37.7 to 44.4 g dry weight  $M^{-1}$  succinate and were not greatly affected by dilution rates or oxygen concentrations. The  $C_4$  dicarboxylic acids succinate, malate and fumarate induced the highest respiration rates in all of the steady states where  $(NH_4)_2SO_4$  was 1.0 g  $L^{-1}$ . The amino acids aspartate, asparagine and glutamate could also be used as respiratory substrates, especially when  $(NH_4)_2SO_4$  was decreased to 0.1 g  $L^{-1}$ . Glutamine-dependent respiration was seen only when  $(NH_4)_2SO_4$  was 0.1 g  $L^{-1}$  and therefore appears to be under tight ammonia control. Nitrogenase activity was detected when the culture was switched from a succinate-limited steady-state to an oxygen-limited steady-state and was stimulated by myoglobin. The total polypeptides of cells from three steady states that differed in ammonia and oxygen concentrations were compared by two-dimensional gel electrophoresis from three steady states that differed in ammonia and oxygen concentrations. Nearly 60% of the resolved polypeptides were affected by oxygen and 20% were affected by ammonia. These data taken together with other data indicate that oxygen has a major influence on the polypeptides of *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20 but does not singularly affect morphological changes that are associated with bacteroids.

A significant finding in our study was that aspartate, asparagine, glutamate and glutamine could all induce oxygen uptake by strain 3G4b20. Aspartate, which is structurally similar to succinate, was used in all of the steady-state conditions tested, especially when dissolved oxygen was greater than 2.3 micromoles and  $(NH_4)_2SO_4$  was 0.1 g  $L^{-1}$ . Glutamine did not induce oxygen uptake at any steady state tested where  $(NH_4)_2SO_4$  was 1.0 g  $L^{-1}$ . However, glutamine became a good substrate when  $(NH_4)_2SO_4$  was lowered to 0.1 g  $L^{-1}$ . This shows that  $(NH_4)_2SO_4$  exerts tight control over the use of glutamine as a respiratory substrate. Asparagine and glutamate-induced respiration showed similar patterns and seemed to be influenced by dilution rate. At lower dilution rates ( $D = 0.03$ ) activity was 15-20 nmols  $min^{-1}mg^{-1}$  protein. When  $(NH_4)_2SO_4$  was lowered to 0.1 g  $L^{-1}$ , asparagine-dependent oxygen uptake exceeded succinate and was higher than any substrate tested. Under these conditions

(steady-state) the amino acids had respiratory activity that was equal to or greater than the C<sub>4</sub> dicarboxylic acids fumarate, malate and succinate. From these data it can be concluded that (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration can exert another level of general respiratory control on succinate-limited *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20 that had not previously been seen.

It is most important to note that these studies using an artificial model system gave results very similar to those field and greenhouse studies summarized earlier in this report. Thus, continuation and improvement of this approach encourages us to believe that we can thus identify more fully the regulatory compounds and environment needed to optimize nitrogen fixation in peanut. Elucidation of this process will allow the peanut breeder to specifically engineer the cultivars to allow maximum BNF.

### The Effect of Plant Passage on Nitrogen Fixation by Peanut Rhizobia

The second question posed in the beginning of this section is whether plant passage affects the stability of the nitrogen fixation property in peanut rhizobia. Plant passage occurs when an inoculum infects a peanut plant, forms nodules and then when the plant is harvested the bacteria are released in the soil ready for an additional cycle.

High nitrogen fixation rates can be achieved in symbiotic systems when the most effective *Rhizobium* infects the roots of the most productive plant genotype. This brings about the possibility of enhancing biological nitrogen fixation by selection procedures. Historically, attempts to optimize the *Rhizobium*-legume symbiosis have put most of the emphasis on the bacterium rather than the host plant. This approach has been unsuccessful in achieving further significant increases in crop yield.

Plant passage is an alternative to the typical selection procedure for effective rhizobial cells. This selection approach combines the selective properties of the host plant with the intrinsic variability in nitrogen fixation effectiveness of *Rhizobium* isolates. The present investigation examines the significance of plant passage as a method for selection of *Bradyrhizobium* isolates with increased symbiotic effectiveness on *A. hypogaea* genotypes.

The effects of four individual plant passage selection cycles of *Bradyrhizobium* sp. on nitrogen fixation traits in two *A. hypogaea* cultivars are summarized in Tables 3 and 4. Each value represents the net contribution of a particular plant passage pool isolate to the symbiotic effectiveness as compared with its respective original *Bradyrhizobium* sp. strain.

The plant passage isolates derived from *Bradyrhizobium* sp. strain 3G4b20 significantly increased the total nitrogen and plant shoot dry weight over all selection cycles (PP2 PP3 PP4) in both cultivars Florigiant and Argentine. In general, these increases were not correlated with nodule fresh weight, nodule number or nitrogenase activity except selection cycle two (PP2) where significant increases in nodule fresh weight and nitrogenase activity were observed in both cultivars. In selection cycle three (PP3) the cultivar Florigiant and strain 3G4b20 combination resulted in a significant increase in nodule fresh weight, total nitrogen and plant shoot dry weight.

Table 3. Effect of plant passage (PP) expressed as a difference between treatment mean of plant passage isolates and original Bradyrhizobium strains in the individual selection cycles on cultivar Argentine

Plant passage cycle	Strain	Inoculum source	Total nitrogen (mg/plant)	Plant shoot dry weight (g/plant)	Nodule number	Nodule weight (g/plant)	Nitrogenase activity (uM C <sub>2</sub> H <sub>4</sub> /hr/pl)
PP2	3G4b20	Original	71.51	2.44	NO	0.59	7.91
		PP1	58.05*	2.02*	NO	0.34*	7.20*
	CB756	Original	45.97	2.52	NO	0.61	6.94
		PP1	30.39*	0.78*	NO	0.73*	4.97*
	3G4b5	Original	18.89	0.69	NO	0.13	5.75
		PP1	2.10	0.15	NO	0.05	2.01
LSD(p = 0.05)			25.50	0.75	--	0.34	3.31
PP3	3G4b20	Original	162.70	5.42	198.75	1.24	46.60
		PP2	73.50*	1.86	23.75	0.38	1.51
	CB756	Original	169.64	6.01	194.00	1.26	44.29
		PP2	40.19	0.56	56.75	0.47	11.00
	3G4b5	Original	84.87	3.31	128.25	0.97	25.21
		PP2	85.40*	2.26*	47.50	0.55	17.05
LSD(p = 0.05)			68.52	1.88	63.83	0.57	18.87
PP4	3G4b20	Original	203.29	8.00	119.50	1.25	46.15
		PP3	68.96	2.37*	50.00	0.08	4.75
	CB756	Original	247.76	8.87	220.50	1.42	66.07
		PP3	14.08	1.31	-2.00	0.24	-4.07
	3G4b5	Original	27.75	1.07	59.00	0.32	21.27
		PP3	24.89	0.71	31.25	0.54	9.25
LSD(p = 0.05)			79.72	2.34	51.07	0.69	20.26

\*Indicates significant difference (LSD p = 0.05). NO = not determined. Original values represent actual treatment mean. PP values represent the difference.

Table 4. Effect of plant passage (PP) expressed as a difference between treatment mean of plant passage isolates and original Bradyrhizobium strains in the individual selection cycles on cultivar Florigiant

Plant passage cycle	Strain	Inoculum source	Total nitrogen (mg/plant)	Plant shoot dry weight (g/plant)	Nodule number	Nodule weight (g/plant)	Nitrogenase activity (uM C <sub>2</sub> H <sub>4</sub> /hr/pl)
PP2	3G4b20	Original	103.16	4.03	NO	1.79	12.29
		PP1	56.06*	0.78*	NO	0.27	3.41*
	CB756	Original	77.03	4.05	NO	1.93	8.05
		PP1	27.47*	0.76	NO	0.58*	5.87*
	3G4b5	Original	14.67	1.17	NO	0.60	5.07
		PP1	15.54	0.41	NO	0.13	1.52
LSD(p = 0.05)			25.50	0.75	--	0.34	3.31
PP3	3G4b20	Original	243.12	6.40	166.00	1.46	44.59
		PP2	79.77*	2.28*	43.00	0.66*	11.06
	CB756	Original	200.31	7.34	233.35	2.13	65.29
		PP2	75.66*	0.58	50.75	0.55	4.59
	3G4b5	Original	165.62	5.64	240.75	1.93	50.62
		PP2	47.63	0.18	2.00	0.92*	8.38
LSD(p = 0.05)			68.52	1.88	63.83	0.57	18.87
PP4	3G4b20	Original	281.93	11.05	156.00	1.98	50.52
		PP3	129.44*	4.91*	34.75	0.68	29.60*
	CB756	Original	401.76	14.44	231.50	2.37	76.77
		PP3	80.02*	2.16	88.50*	1.19	1.95
	3G4b5	Original	107.39	2.91	172.75	1.27	20.58
		PP3	39.64	1.89	39.25	0.56	8.85
LSD(p = 0.05)			79.72	2.34	51.07	0.69	20.26

\*Indicates significant difference (LSD p = 0.05). NO = not determined. Original values represent actual treatment mean. PP values represent the difference.

Similar results were observed for the plant passage isolates derived from *Bradyrhizobium* sp. strain CB756. In selection cycle two (PP2) all nitrogen fixation traits tested were significantly different from the original strains, indicating a positive effect of the plant passage pooled inoculum towards the overall symbiotic effectiveness. However, in the subsequent cycles (PP3 PP4) the positive contribution of the plant passage pooled inoculum was restricted to total nitrogen and plant shoot dry weight in cultivar Florigiant.

The symbiotic effectiveness of each plant passage pool was tested in a combined study using cultivars Florigiant and Argentine. The overall objective was to remove the seasonal effect on plant yield observed in the individual selection cycles. In general, the results obtained in the combined study were similar to those obtained in the individual selection cycle except that most of the variations due to plant passage isolates in the combined study were confined to cv. Florigiant. The plant passage isolates from strains 3G4b20 and CB756 significantly increased the total nitrogen and plant shoot dry weight, whereas no significant changes were observed for the plant passage isolates from strain 3G4b5.

The significant changes observed with strains 3G4b20 and CB756 were highly correlated with the harvesting date. Most of the significant increases in total nitrogen and plant shoot dry weight for strain 3G4b20 were expressed between 8 and 10 weeks (harvests 2 and 3), whereas those increases due to plant passage isolates from strain CB756 were expressed between 4 and 8 weeks after planting (harvests 1 and 2). Generally, no significant changes were observed in nodule number or nodule fresh weight. Strain 3G4b5 did not significantly change the overall effectiveness for all nitrogen fixation traits evaluated.

The results of analysis of variance by harvest dates are shown in Tables 5 and 6. Significant differences in total nitrogen and plant shoot dry weight were found associated with the cultivar, strain, inoculum source (PP) and the interaction of cultivar x strain at each harvest date. The contrasts set up for the inoculum sources (plant passage versus original strains) averaged over cultivars and strains indicated a significant effect of plant passage selection cycles on total nitrogen and plant shoot dry weight as compared with the original strains. Significant cultivar-dependent variations in nodule number and nodule fresh weight were found at each harvest, along with some significant effects due to strain, inoculum and the cultivar x strain interaction. The contrasts for inoculum source versus original strains averaged over cultivars and strains show a positive effect for nodule number from PP4 and PP5 at 8 weeks (harvest 2) only.

The significant differences in total nitrogen and plant shoot dry weight found for the interaction between cultivar and strain at 4 and 8 weeks after planting (harvests 1 and 2) indicates a differential response of the two cultivars and the strains utilized. The strains CB756 and 3G4b20, including the original and their plant passage isolates, performed differentially in each cultivar at different harvest dates. Both strains yielded more nitrogen and shoot material when associated with cv. Florigiant. Strain CB756 accumulated more nitrogen and plant shoot material at harvest 1 (4 weeks) than strain 3G4b20. Following harvest 2 (8 weeks) the strain 3G4b20 accumulated more nitrogen and plant shoot material than strain CB756.



Table 5. Mean squares of analysis of variance of combined plant passage study for nodule number and nodule fresh weight.

Source	df	-----Nodule number-----			----Nodule fresh weight----		
		HV1	HV2	HV3	HV1	HV2	HV3
Block	03	2517.58	3970.05	27670.10	0.20	0.02	1.59
Cultivar (C)	01	49572.67**	125194.80**	153081.63**	6.76**	32.63**	51.91**
Strain (S)	02	6764.30**	6050.65	25508.93	0.09	0.77*	4.11**
C x S	02	2104.37	27720.22**	5972.23	0.07	1.70**	0.88
Plant passage (PP)	04	2329.94	7322.42*	1365.05	0.04	0.41	0.60
C x PP	04	506.69	1092.13	3193.82	0.01	0.31	0.16
S x PP	08	451.30	3639.98	2152.64	0.04	0.25	0.17
C x S x PP	08	2112.39*	1692.27	2107.29	0.07	0.11	0.70
Error	87	1024.10	2488.10	5836.19	0.04	0.22	0.56
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<u>Contrasts for plant passage</u>							
Original vs. PP2	01	1045.33	6340.00	1111.68	0.02	0.69	0.33
Original vs. PP3	01	99.18	5940.75	2465.33	0.01	0.03	0.18
Original vs. PP4	01	1160.33	21378.52**	4820.02	0.03	1.05*	0.56
Original vs. PP5	01	2745.18	21252.08**	2640.33	0.05	0.73	2.25*

\*,\*\*Indicate significance at  $p = 0.05$  and  $0.01$ , respectively. HV1, HV2 and HV3 represent harvesting dates at 4, 8 and 10 weeks after planting, respectively.

Table 6. Mean squares of analysis of variance of combined plant passage study for total nitrogen and plant dry weight

Source	df	-----Nodule number-----			----Nodule fresh weight----		
		HV1	HV2	HV3	HV1	HV2	HV3
Block	03	614.08	2869.45	44195.56	0.41	2.30	12.16
Cultivar (C)	01	40172.89**	228406.54**	203020.97**	25.51**	139.53**	104.53**
Strain (C)	02	19471.65**	419449.64**	878781.10**	7.42**	161.80**	653.73**
C x S	02	2841.57**	30592.43**	1308.57	1.10**	22.46**	8.86
Plant passage (PP)	04	2282.85**	11443.19**	25260.27**	0.92**	7.88*	23.84**
C x PP	04	1250.98*	3446.81	14332.97	0.49	2.24	11.28
S x PP	08	326.43	3010.88	10685.64	0.29	3.43	9.64
C x S x PP	08	541.53	2765.18	8168.39	0.30	1.58	6.48
Error	87	386.54	3276.23	6817.39	0.23	2.36	6.42
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<u>Contrasts for plant passage</u>							
Original vs. PP2	01	3252.66**	32487.53**	62324.69**	1.41*	20.65**	46.78**
Original vs. PP3	01	7695.00**	21856.00*	51944.49**	3.18**	23.49**	57.15**
Original vs. PP4	01	5687.41**	27287.74**	78262.09**	2.24**	10.69*	78.00**
Original vs. PP5	01	2228.23*	30400.83**	21882.89*	1.36*	18.42**	45.31**

\*, \*\*Indicate significance at  $p = 0.05$  and  $0.01$ , respectively. HV1, HV2 and HV3 represent harvesting dates at 4, 8 and 10 weeks after planting, respectively.

The plant passage data may be summarized as follows:

1. Significant changes in symbiotic effectiveness of *Bradyrhizobium* sp. strains 3G4b20, CB756 and 3G4b5 were observed when these strains were repeatedly inoculated and isolated from the root nodules of peanut cultivars Argentine and Florigiant. The plant passage isolates from strains 3G4b20 and CB756 significantly increased the total nitrogen and plant shoot dry weight while strain 3G4b5 showed no significant changes and even tended to decrease the symbiotic effectiveness with these repeated passages.
2. The significant changes in total nitrogen and plant shoot dry weight were not correlated with nodule number and nodule mass.
3. The observed variations in symbiotic effectiveness were highly dependent on the cultivar considered. Cultivar Florigiant accumulated more nitrogen and yielded more plant material, nodule number and nodule mass than cultivar Argentine. Also, most of the significant increases in total nitrogen and plant shoot dry weight due to the plant passage inocula were confined to cultivar Florigiant.
4. The plant age influenced the expression of symbiotic effectiveness. Most of the significant increases in total nitrogen and plant shoot dry weight due to strain CB756 on cultivar Florigiant were observed between 4 and 8 weeks after planting (harvests 1 and 2). Strain 3G4b20 significantly increased total nitrogen and plant shoot dry weight between 8 and 10 weeks after planting (harvests 2 and 3).
5. It was suggested that the heterogeneity status of the original culture is a possible source of variation in symbiotic effectiveness. The selection pressure exerted by the host plant could explain the overall effect of plant passage.

In this study a series of plant passage isolates of *Bradyrhizobium* strains from each cultivar/strain/plant passage combination were obtained and individually stored. This germplasm represents the inoculum source used in all plant passage cycles and therefore could be invaluable in future studies examining the genetic diversity in these isolates.

## TRAINING OUTPUTS

### Students Completing Their Studies

- George Allen (PhD, 1988). Thesis: Oxygen and succinate regulatory control of *Bradyrhizobium* sp. (*Arachis*) strain 3G4b20 during the shift from aerobic to microaerobic conditions (under the direction of G. H. Elkan).
- Mark Barbour (PhD, 1988). Thesis: Effects of plasmid curing and a plasmid copy number mutant on symbiotic and physiological properties of *Rhizobium fredii* USDA 206 (under the direction of G. H. Elkan).
- Cecilia Bianchi\* (MS, 1988). Thesis: Effect of plant passage through peanuts (*Arachis hypogaea* L.) on competitive ability of *Bradyrhizobium* sp. (under the direction of G. H. Elkan).
- Tulio Cassini\* (PhD, 1988). Thesis: Variation in symbiotic effectiveness of *Bradyrhizobium* sp. through plant passage in peanut (*Arachis hypogaea* L.) (under the direction of G. H. Elkan).
- Daniel Grimm (PhD, 1988). Thesis: Influence of *Bradyrhizobium* sp. on nodule and seed composition in peanut (*Arachis hypogaea* L.) (under the direction of G. H. Elkan).

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\*Stipend from other sources; only research supported by CRSP funds.

**Continuing Students**

Seanne Udell (PhD candidate) (under the direction of G. H. Elkan).  
Terrence Miller (PhD candidate) (under the direction of G. H. Elkan).

**New Students**

William Gillette\* (MS candidate) (under the direction of G. H. Elkan).  
Bruce Urtz\* (PhD candidate) (under the direction of G. H. Elkan).

**PRESENTATIONS****Paper Presented at Annual Meeting of American Microbiology Society, Atlanta, Georgia, March 8-13, 1987**

Jarvis, B. D. W., J. N. Mathis, E. S. Maxwell and G. H. Elkan. Overproduction of *nif* mRNA in a *Bradyrhizobium japonicum* USDA 110 derivative which lacks nitrogen fixation competence.

**Papers Presented at the Annual Meeting of American Peanut Research and Education Society, Orlando, Florida, July 14-17, 1987**

Phillips, T. D., J. C. Wynne, T. J. Schneeweis and G. H. Elkan. Inheritance of symbiotic nitrogen fixation in two peanut crosses.

Wijewickrama, P. J. A., T. J. Schneeweis, J. C. Wynne and G. H. Elkan. Differences in competitive ability of single and mixed-strain inoculants for peanut.

**Papers Presented at XIIth American Rhizobium Conference, Quebec, Canada, August 9-15, 1987**

Allen, G. C. and G. H. Elkan. Effect of varying oxygen concentrations on viability, respiration, and yield of a *Bradyrhizobium* sp. (*Arachis*) strain grown in a succinate-limited chemostat.

Barbour, W. M., T. B. Miller and G. H. Elkan. Effects of plasmid curing on *Rhizobium fredii* USDA 206.

Casas, I. A., T. J. Schneeweis, R. S. Steece, J. C. Wynne and G. H. Elkan. Strain influence on nitrogen fixation during the growth cycle of peanut (*Arachis hypogaea* L.).

Cassini, S. T., I. A. Casas and G. H. Elkan. Root nodule protein profiles during growth cycle of *Arachis hypogaea*.

Elkan, G. H. and M. D. Stowers. Carbohydrate metabolism in the Rhizobiaceae (conference keynote address).

Grimm, D. T., I. A. Casas and G. H. Elkan. Influence of *Bradyrhizobium* sp. on free amino acids in root nodules of peanut (*Arachis hypogaea* L.).

**Paper Presented at XIV Latin American Nitrogen Fixation Conference, Santiago, Chile, November 21-24, 1988**

Bianchi, C. and G. H. Elkan. Competitive ability of isolates of *Bradyrhizobium* that had undergone plant passage through plants of peanut (*Arachis hypogaea* L.).

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#### TECHNOLOGY TRANSFER

A number of useful results are occurring for the USA as a result of the CRSP-supported research from the soil microbiology project.

1. *Rhizobium* strains from the tropics, which were screened for domestic use, have been incorporated into commercial inoculum for peanuts by the major inoculum producer in the U.S.
2. We have signed a contract with a biotechnology firm (1988) interested in greenhouse and field testing of additional peanut rhizobia for the purpose of developing inocula for commercial use.
3. A major peanut product producer has recently contacted us for the purpose of pursuing research in the modification of peanut seed composition through the use of our *Rhizobium* strains.

In each of these cases the companies involved have been given *nonexclusive* use of the *Rhizobium* strains since the studies reported used federal funds and results are in the public domain.

The reason that we are discussing domestic technology transfer is to emphasize that the Peanut CRSP research is beneficial domestically. The peanut breeding program, too, has been able to produce efficient nitrogen-fixing cultivars for domestic use as a spin-off from CRSP-supported research.

#### PLANS FOR 1989

The studies reported herein are on-going and actually we have just reached a point where we can start to "breed" the rhizobia for increased efficiency and competitiveness. We are identifying the factors needed by the plant breeders for exploiting the cultivar-*Rhizobium* interactions, so we plan to work more closely in conjunction with the breeding project.

Since this project has been recommended for phase-down, realistically plans for 1989 are contingent upon funding. All overseas funding for this work has been discontinued at a point; unfortunately, when we had begun to understand the lack of response to *Rhizobium* inoculation in Thailand (trace element deficiency, especially molybdenum), we would think it important to continue the overseas studies.

If funds for overseas work are restored to any level and, having determined the need for trace elements as a precondition for effective nitrogen fixation, we would continue HC work with

continuing objectives as well as objectives revised as a result of the CRSP conference at Khon Kaen in 1986:

1. Continue to determine constraints to BNF (*i.e.*, carefully elucidate the need for trace elements).
2. Evaluate the need for inoculation in local field tests.
3. Screen and identify rhizobia effective with local and advanced introduced cultivars in collaboration with the peanut breeders.
4. Develop cultivars for increased BNF with interaction with breeders.
5. Develop inoculant technology that will enhance *Bradyrhizobium* effectiveness and competitiveness.
6. Screen and identify effective rhizobial strains tolerant for adverse conditions (*i.e.*, flooding, drought, acid, alkali, etc.).

These objectives are based on the following questions which resulted from the initial research:

1. What is peanut yield potential, at present, under optimum conditions of fertility and disease control; (a) in the different soil types, and (b) under different farming regimes?

Second priority: 1) Wet season rainfall  
 Highest priority: 2) Dry season rainfall  
 3) Dry season irrigated

2. How much nitrogen does it take to reach this potential (do total nitrogen in tops, roots, pods, x yield)?
3. With indigenous rhizobia, how much of the nitrogen requirement is supplied (P + K + minor elements + integrated pest management)?
4. What is the minimum input needed to obtain near optimum yield (as in question 1)? (This was really the starting point for the CRSP since we needed to maximize yield with minimum input using breeding, disease and insect control and BNF.)
5. Determine available N in soil by use of non-nod peanut (measure total N x yield after yellowing is severe). How much soil nitrogen is available?
6. Select cultivars x strains in greenhouse tests and then field tests (how can we maximize N fixation)?

#### DOMESTIC RESEARCH PLANS

CRSP research has resulted in several significant observations which will form the core of future research. These are (1) *Rhizobium* strain NC92 from NCSU gives consistently higher yields with some cultivars of peanuts, yet this is not due to increased total nitrogen fixation; (2) rhizobia can modify composition of peanut components (*i.e.*, free amino acids, fatty acids); (3) even if nitrogen-fixing capability is genetically enhanced, increased symbiotic nitrogen fixation is not increased because of the regulatory role played by the host peanut; (4) field studies with peanut rhizobia are difficult because traditional serological techniques are not as accurate as with other rhizobia. Research plans for the immediate future, pending additional funding, include the following:

1. Continue to isolate, test and make available to host country collaborators promising strains of rhizobia.

2. Continue to collaborate with the breeding project to select peanut cultivars for high nitrogen-fixing capability and to develop screening techniques for more easily identifying such germplasm.

3. To improve field identification techniques by adapting enzyme-linked immunosorbent assay (ELISA) and monoclonal assays for precise *Rhizobium* identification for competition studies.

4. Develop alternative inoculation techniques to make the introduced *Rhizobium* more competitive against the indigenous, less efficient rhizobia.

5. To determine the mechanism of action of the cultivar-*Rhizobium* interaction involving NC92 causing enhanced peanut yield—

- A gene library of NC92 mutants has been prepared and will be screened to isolate the active factor(s).
- Peanut tissue culture techniques are being designed to allow us to determine the symbiotic regulatory compounds (nodulins) produced by the plant host as mediated by NC92.
- Using the “artificial nodule” technique to determine the symbiotic regulatory compounds produced by NC92 pseudobacteroids (bacteroidins).



TX/SM/TP  
Influence of Soil Microbiology on Nitrogen Fixation  
and Growth of Peanut in Thailand and the Philippines  
B. Mycorrhizal Considerations

Texas A&M University—Thailand and Philippines  
Ruth Ann Taber, Principal Investigator, TAMU

## INTRODUCTION

Mycorrhizal fungi inhabit the roots of almost all terrestrial plants, including important crop plants such as peanut. Mycorrhizal fungi aid plant growth by functioning as accessory roots. In some species these fungi have been shown to promote solubility and uptake of minerals (especially phosphorus); protect plants roots from disease; produce growth-promoting hormones; increase salt, drought, and flooding tolerance; and may act synergistically with *Rhizobium* on legumes. The relative efficiencies of these fungi on peanut are relatively unknown. Endomycorrhizal fungi have been reported in peanut roots—in Texas five species representing 3 genera (*Glomus*, *Gigaspora*, and *Sclerocystis*) are recognized as associative with peanut, although their value has never been assessed. A better understanding of the various endomycorrhizal fungi present in the roots of peanut both in the LDC's and in peanut producing states in the U.S. is urgently needed. This report covers our progress from 1 July 1987 to 30 June 1988.

## MAJOR ACCOMPLISHMENTS

Convincing evidence has been accumulated that documents the beneficial effects of mycorrhizal fungi on peanut growth. Differences in efficiencies of various species have been demonstrated. Although percent root colonization by two different species at harvest was significantly different, the two different species effected over 300% increases in kernel weights (yield) in greenhouse tests. Use of two fungicides as seed treatments did not influence colonization rates. Over twice as much phosphorus was detected in mycorrhizal plants as in the controls. A threefold increase in manganese in leaf tissue of inoculated plants was observed. One mycorrhizal fungus *Glomus intraradices*, was effective in nullifying the detrimental effects of the root knot nematodes. The fungus inhibited the development of the nematodes into adults. In field tests at Yoakum, inocula of *Glomus deserticola* at two different rates resulted in greater yield of cv. 'Florunner' than those of the controls. Several publications were finalized in the Philippines and Thailand with Southeast Asian collaborative scientists. Arrangements were finalized for Mr. Randy Garber to develop monoclonal antibodies against *Glomus* sp. at the USDA Beckley, West Virginia laboratory. Monoclonal antibodies will be used in his research to identify species.

## EXPECTED IMPACT OF THE PROJECT

**In host country**—An increased understanding of these beneficial fungi should lead to improved peanut growth and yield in LDC's. Utilization of appropriate, efficient strains of these fungi should allow for peanut plantings in the more arid regions, in areas where soil fertility is low, and increase the value of peanut in intercropping sequence. The demonstration of their beneficial interactions with *Rhizobium* species on other crops holds promise that they may be exploited for similar interactions on peanut.

**In the United States**—Knowledge of efficient mycorrhizal fungi, access to untested strains, and methodology developed as a result of this project should lead to development of inoculation procedures to assure the presence of appropriate fungi on peanut to obtain maximum yields. In addition, discovery of mycorrhizal strains adapted to soils with high salt content, low water potential, or flooded conditions could help farmers use land currently unsuitable for peanut growth.

## GOAL

To increase peanut yield/unit area in the LDC's and the U.S.A. through manipulation of mycorrhizal fungi and *Bradyrhizobium* in peanut roots and to bring into production acreages idle because of lack of sufficient water, high salts in the soils, or flooding conditions.

## OBJECTIVES

- A. The overall objective is to help maximize peanut production in each country through manipulation of the microbial inhabitants of the root.
- B. Conduct a collaborative survey of endomycorrhizal fungi predominant in the rhizosphere of peanuts growing in the U.S. and LDC's.
- C. To establish a collection of mycorrhizal fungi in pot culture, develop inoculation techniques, and field test various mycorrhizal isolates.
- D. Establish the effectiveness of selected mycorrhizal fungi for alleviating salinity, drought, and flooding stress in peanut.
- E. Establish the effectiveness of selected mycorrhizal species for increased uptake of phosphorus.
- F. Determine whether mycorrhizal fungi can afford the peanut protection against soil-borne diseases.
- G. Determine the effectiveness of mixed rhizobia and mycorrhizal fungi for increased P uptake and other synergistic relationships.

## ORGANIZATION

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 Mr. Randy Garber, Ph.D. Candidate, College Station

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## ACCOMPLISHMENTS IN DETAIL

## UNITED STATES

1. Effect of *Glomus etunicatum* and *G. deserticola* on Tamnut 74 yield and elemental uptake.

## Greenhouse Yield Test

Pasteurized soil mix (1:1 Brazos river soil:river sand) was infested with either *G. etunicatum* or *G. deserticola* in plastic-lined one-half bushel baskets. A non-inoculated soil mix served as control soil. All soils were inoculated with granular *Bradyrhizobium* obtained from the Nitragin Company, Milwaukee, Wisconsin. Three seeds of Tamnut-74 were planted per basket. The experiment was a randomized complete block design replicated four times. Each basket received 40 ml (2.6 g available phosphoric acid) Osmocote 30 days after planting. Plants were harvested at 120 days.

Colonization of peanut roots by the two fungi differed—*G. etunicatum* colonized 2.5x more root systems than did *G. deserticola*. Dry top weights of *G. etunicatum* plants were 243% greater than those of the controls and plants inoculated with *G. deserticola* were 250% greater. Pod numbers, pod weights, and kernel weights from mycorrhizal plants exceeded 200% of those from control plants. Kernel weights from plants inoculated with either species were over 300% more than those from control plants. By 80 days a threefold increase in manganese in leaf tissue was observed in *G. etunicatum* plants. Over twice as much phosphorus was found in mycorrhizal plants as in the controls. See Tables 1 and 2.

Response of peanut plants did not parallel the level of colonization. It is evident that levels of colonization (at harvest) did not necessarily coincide with higher nutrient levels observed earlier in the season.

Table 1. Effect of *Glomus etunicatum* and *G. deserticola* on peanuts cv. 'Tamnut 74'. Greenhouse study.

	<i>G. etunicatum</i>	<i>G. deserticola</i>	Control
Dry Top Wt. (grams)	66.0 a*	70.5 a	28.2 b
% Colonized	16.8 a	6.8 b	.0 c
Pod No.	65.4 a	81.0 b	29.6 c
Pod Wt. (grams)	61.2 a	70.7 a	23.7 b
Kernel Wt.	34.2 a	40.5 a	10.8 b

\* Means in each line with the same letter are not significantly different according to Duncan's multiple range test ( $p = .05$ ).

**Table 2. Effect of *G. etunicatum* and *G. deserticola* on elemental uptake. Leaf tissue analysis (parts per million). Greenhouse study.**

	<i>Glomus etunicatum</i>		<i>Glomus deserticola</i>		Control	
	40 days	80 days	40 days	80 days	40 days	80 days
Phosphorus	2265	4150a*	2691	3848a	1516	1897 b
Magnesium	3932	3807a	4508	3931a	3834	2786 b
Nitrogen	--	76a	--	76a	--	59 b
Calcium	13967	14456a	13837	9150a	21347	8426a
Manganese	49	198a	52	98 b	59	60 b

\* Means in each line with the same letter are not significantly different according to Duncan's multiple range test ( $p = .05$ ).

## 2. Effect of two fungicides on growth and colonization of roots of peanut cv. 'Tamnut-74' by *G. etunicatum*.

### Fungicide Seed Treatment

Tamnut-74 peanut seed were treated (0.2 gm fungicide per 16 seeds) with either Ardsan 50 red, Granox P-F-M, or were left untreated. Pasteurized soil in twenty-four 15 cm pots was infested with granular *Bradyrhizobium*. Soil in half of the pots was also infested with *Glomus etunicatum* and planted with 1 seed per pot with four replications per treatment. Plants were harvested at 50 days.

Fungicide seed treatment had no apparent effect on the colonization level at 50 days. Non-mycorrhizal plants exhibited distinct stunting. See Table 3 below.

**Table 3. Effect of Ardsan 50 red and Granox P-F-M on growth and colonization of roots of peanut cv. 'Tamnut-74' by *G. etunicatum*. Greenhouse study.**

Fungus	Fungicide	Shoot/Root Ratio	Root Length (cm)	% Root Length Colonized
+	G*	2.8 ± 0.4	2661 ± 461	39.8 ± 13.9
-	G*	2.2 ± 0.6	1984 ± 789	0
+	A**	4.3 ± 0.8	2909 ± 1640	37.4 ± 21.5
-	A**	2.4 ± 0.4	2087 ± 454	0
+	C***	3.0 ± 0.8	2351 ± 1203	41.3 ± 32.5
-	C***	2.3 ± 0.8	2188 ± 266	0

+ *Glomus etunicatum*  
 - non-mycorrhizal  
 · Granox  
 \*\* Ardsan  
 \*\*\* non-fungicide control

### 3. Effect of phosphorus, *Glomus etunicatum*, and *Bradyrhizobium* on Tamnut -74.

#### Low Phosphorus Nutrient Test.

The effect of available phosphorus on mycorrhizal colonization of peanut cv. 'Tamnut-74' was tested using a sand hydroponic system with Hoagland's nutrient solution modified by varying the P levels. The experimental treatments included: 4 phosphorus levels, *Bradyrhizobium* and non-*Bradyrhizobium* inoculation, and mycorrhizal inoculation (*Glomus etunicatum*) or no mycorrhizal inoculation, in all combinations. The main treatment was the *Bradyrhizobium*/non-*Bradyrhizobium* treatment in a split block design with four replications with one plant per pot. Plants were grown in 25 cm pots and were harvested at 40 and 80 days.

At the 40 day harvest, mycorrhizal/rhizobium plants had higher shoot/root ratios at the lower three levels of available phosphorus than at 6.25 ppm. The inoculated mycorrhizal/rhizobium plants produced greater top weights. The level of colonization at 6.25 ppm P was significantly lower. Roots on non-mycorrhizal *Bradyrhizobium* plants were longer than other root systems. Acetylene reduction AR values at 40 days were also higher in the non-mycorrhizal plants while the number of nodules showed the opposite trend, i.e. mycorrhizal plants had a higher number of nodules (Tables 4 and 5). At 80 days, similar trends for AR's and nodule number were seen at the lower three phosphorus levels. At the same phosphorus levels at 80 days, the shoot/root ratios indicate that the mycorrhizal/rhizobium treatment supported more shoot biomass. (Tables 6 and 7). At both 40 and 80 days the non-rhizobium plants showed considerable stunting and a general lack of vigor. It is apparent from this test that shifts in biomass accumulation and distribution were occurring. Based on AR values and number of nodules, the nodulation process and efficiency were affected by the mycorrhizal treatments.

Table 4. Effect of phosphorus, *Glomus etunicatum* and *Bradyrhizobium* on nodule formation and growth of Tamnut-74. *Bradyrhizobium* added. 40 days. Greenhouse study.

P Level	Fungus	Shoot/ Root Ratio	Root Length (cm)	% root Length Colonized	AR <sup>1</sup> nmol/mg/hr	Nodule No.
0.05	+	6.2 ± 1.9	1485 ± 598	10.1	938 ± 516	75 ± 24
0.05	-	3.0 ± 0.35	2184 ± 479	0	3227 ± 1608	33 ± 16
0.25	+	5.3 ± 1.6	1342 ± 781	9.8	1009 ± 527	64 ± 30
0.25	-	3.8 ± 1.6	1773 ± 781	0	2833 ± 1301	33 ± 14
1.25	+	6.4 ± 1.0	1355 ± 645	8.6	614 ± 40	73 ± 45
1.25	-	3.6 ± 1.1	2222 ± 933	0	1260 ± 194	52 ± 4
6.25	+	6.9 ± 0.9	1175 ± 374	0.9	325 ± 0.9	98 ± 34
6.25	-	7.0 ± 2.0	1272 ± 668	0	527 ± 279~	66 ± 19

<sup>1</sup>Acetylene reduction.

**Table 5.** Effect of phosphorus level and *G. etunicatum* on shoot/root ratio of peanut cv. 'Tannut-74'. No *Bradyrhizobium* inoculum. 40 days. Greenhouse study.

Phosphorus Level	Fungus	Shoot/Root Ratio
0.05	+	1.9 ± 0.1
0.05	-	1.5 ± 0.3
0.25	-	2.2 ± 0.3
0.25	-	1.3 ± 0.2
1.25	+	2.4 ± 0.2
1.25	-	1.7 ± 0.2
6.25	+	3.0 ± 0.5
6.25	-	2.5 ± 0.5

**Table 6.** Effect of phosphorus level and *Glomus etunicatum* on shoot/root ratio of peanut cv. 'Tannut-74'. *Bradyrhizobium* added. 80 days. Greenhouse study.

Phosphorus Level	Fungus	Shoot/Root Ratio	AR <sup>1</sup> nmol/mg/hr	Nodule No.
0.05	+	16.8 ± 3.6	552 ± 112	148 ± 35
0.05	-	6.1 ± 2.0	4876 ± 2502	29 ± 18
0.25	+	21.4 ± 6.1	288 ± 52	163 ± 116
0.25	-	6.6 ± 1.8	3296 ± 994	33 ± 6
1.25	+	21.6 ± 2.4	158 ± 28	274 ± 24
1.25	-	13.7 ± 3.0	379 ± 111	131 ± 35
6.25	+	21.7 ± 3.7	123 ± 25	274 ± 29
6.25	-	28.0 ± 11.7	115 ± 45	285 ± 32

<sup>1</sup>Acetylene reduction.

**Table 7.** Effect of phosphorus level and *Glomus etunicatum* on shoot/root ratio of peanut cv. 'Tamnut-74'. No *Bradyrhizobium* added. 80 days. Greenhouse study.

Phosphorus Level	Fungus	Shoot/Root Ratio
0.05	+	8.0 ± 1.4
0.05	-	5.4 ± 3.4
0.25	+	10.8 ± 8.0
0.25	-	5.0 ± 1.3
1.25	+	11.2 ± 2.5
1.25	-	5.7 ± 1.7
6.25	+	10.7 ± 2.6
6.25	-	6.2 ± 1.3

In the greenhouse yield tests, fungicide test, and the available phosphorus tests, there is good evidence for indicating that peanuts respond positively to mycorrhizal development overall. Numerous explanations may be postulated. The shifts in shoot/root ratios and nodulation activity/development suggest that at least in part, the responses seen may be due to a basic metabolic shift. Hormonal and/or enzymatic patterns may be occurring. The mycorrhizal fungus may be eliciting (at least initially) a shift in growth development similar to an induced pathogen response.

Ongoing tests are designed to determine possible shifts in protein patterns and flavonoid patterns in response to mycorrhizal development. Tissue samples from the available phosphorus tests are being digested and analyzed for phosphorus and nitrogen to gather insight into nutrient accumulation and distribution in mycorrhizal and non-mycorrhizal peanut plants grown in a controlled environment. This information will be incorporated into the Ph.D. dissertation of J. Stephen Neck.

#### 4. Determination of the critical phosphorus level for growth of peanut cv. 'Starr'.

Phosphorus is the most important nutrient involved in the increased growth of mycorrhizal plants and is usually found at higher levels in mycorrhizal plants. The magnitude of the mycorrhizal growth response depends upon the available P present in the soil. The greatest increase in growth usually occurs in soils which are so deficient in P that non-mycorrhizal plants will barely survive.

It was previously determined that peanut cultivars 'Tamnut-74' and 'Florunner' grown in sand culture failed to respond to inoculation with *Glomus etunicatum* when a constant P level of 6.25 ppm was maintained. No significant differences in shoot or root dry weights were detected between mycorrhizal and non-mycorrhizal plants.

A review of the peanut physiology literature failed to reveal any clear-cut concentration at which P becomes deficient for peanut plant growth. Critical values ranged from 1 to 16 ppm depending upon the soil type and the extractant employed (Table 8). Therefore, it was necessary to conduct a preliminary experiment to delineate the concentration at which available P becomes deficient using the sand culture system which will be employed in future experiments. If increased P uptake is the key factor in mycorrhizal stimulation of plant growth, the maximum response to mycorrhizal colonization should occur when P is applied in amounts insufficient for normal plant growth.

**Table 8. Summary of phosphorus level critical for normal peanut growth.**

EXTRACTANT	COMPOSITION	CRITICAL P LEVEL (ppm)
Double Acid	0.15 NHCl + .025 NH <sub>2</sub> SO <sub>4</sub>	4
Bray 1	0.025 N HCl + 0.03 N NH <sub>4</sub> F	7
Bray 2	0.1 N HCl + 0.03 N NH <sub>4</sub> F	5
Olsen	0.5 N NaHCO <sub>3</sub>	10
Oxalate	Oxalic acid + potassium oxalate	1
Lactate	Ammonium lactate + acetic acid	16

Values listed in this table were compiled from Liming, Fertilization and Mineral Nutrition. In Peanut Science and Technology Chapter 6. H. E. Pattee and C. T. Young editors.

A greenhouse study in which 3 seeds of peanut cultivar 'Starr' were planted in 25 cm plastic pots containing coarse drilling sand was initiated. Pots were watered with equal amounts of distilled water every other day for 12 days. At this time seedlings were thinned to 1 healthy seedling per pot and application of nutrient solutions was initiated. Treatments consisted of one-half strength Hoagland's solution adjusted to pH 6.25, containing either 4.0, 1.4, 1.0, 0.5, 0.1, 0.05, 0.005 or 0.0 ppm P. Pots were arranged on the greenhouse bench in a completely randomized design with each treatment being replicated three times. Each pot received 1 l of the appropriate nutrient solution every 5 days for 35 days. Long day conditions were maintained by extending the photoperiod to 12 hours using Gro-lux fluorescent lamps. Plants were observed every other day for visible symptoms of P deficiency. Thirty-six days after initiation of P treatments all plants were harvested. Plant heights, number of leaves, number of branches, number of pegs, shoot and root fresh weight and leaf areas were recorded. Shoots and roots were then dried at 75°C for 72 hours in a Precision Scientific model 645 drying oven and dry weights were recorded.

Deficiency symptoms were first observed 27 days after seedling emergence. Stems of plants receiving 4.0 ppm of P or less demonstrated purpling of stems and visible stunting was observed in plants receiving less than 2.5 ppm P. The lower 3-4 leaves of plants receiving 0.0, 0.005, 0.01, 0.05, and 0.10 ppm P were chlorotic and abscission zones were beginning to form on their petioles. Plants receiving 0.5, 1.0 ppm P demonstrated chlorosis of the lowermost 2 leaves, whereas plants receiving 2.5 or 4.0 ppm P displayed no chlorotic symptoms.

Plant heights and numbers of branches and pegs were least affected by P treatments. The trend was for these parameters to decrease as P levels decreased (Table 9). Leaf area, numbers of leaves, shoot fresh and dry weights, and root fresh and dry weights, demonstrated a much greater response to varying P levels. Leaf areas decreased linearly as P level fell from 4.0 to 1.0 ppm. Phosphorus applied at concentrations below 1.0 ppm resulted in similar leaf area values (Table 8). The number of leaves decreased significantly when P was applied at or below 0.50 ppm. Only slight differences in leaf numbers were observed at P levels below this value. Although plants receiving 1.0 ppm and 2.5 ppm P possessed quite similar leaf numbers, the area per leaf was considerably reduced in plants receiving 1.0 ppm P. This indicates that initiation of P stress does not inhibit leaf formation, but rather limits size of the leaves and results in smaller leaf areas. Fresh and dry weights of both roots and shoots decreased noticeably as the P level fell from 4.0 ppm to 1.0 ppm. At P levels below 1.0 ppm these parameters remained relatively constant (Table 10). Root/shoot ratios increased linearly as P level decreased, indicating that as P became increasingly limiting, less P was available to plant shoots and what little P was absorbed remained there.

Values of the parameters measured indicated available P added at less than 2.5 ppm was insufficient for adequate plant growth. When available P was added at concentrations less than 1.0



ppm severe deficiency symptoms were observed. It is recommended that a P treatment of less than 2.4 ppm be included in future experiments. This will greatly aid in determining if mycorrhizal stimulation of plant growth is primarily due to increased uptake of limited P, or if other factors are involved.

**Table 9. Effect of phosphorus (added as phosphoric acid) on growth parameters of peanut cv. 'Starr'.**

Phosphorus Level ppm	Plant Height cm	Number of Leaves	Leaf Area cm <sup>2</sup>	Number of Branches	Number of Pegs
4.00	17.13	40.00	817.02	6.00	10.00
2.50	14.43	33.00	604.92	5.00	7.33
1.00	12.63	30.00	337.38	4.67	6.00
0.50	10.46	14.33	213.36	2.67	4.33
0.10	10.63	19.00	223.85	3.67	4.33
0.05	9.63	16.33	162.37	4.00	3.67
0.01	10.17	19.67	208.30	2.50	3.00
0.005	10.63	17.00	167.87	3.67	4.67
0.0	11.87	17.33	212.46	4.00	3.33

All values listed are means of three replications.

**Table 10. Effect of phosphorus (added as phosphoric acid) on shoot and root weights of peanut cv. 'Starr'.**

Phosphorus ppm	Shoot Fresh Weight	Root Fresh Weight	Shoot Dry Weight	Root Dry Weight	Root/Shoot
4.0	39.57	19.33	6.17	1.87	0.300
2.5	28.80	21.47	4.27	1.72	0.420
1.0	18.46	15.82	2.80	1.18	0.416
0.50	13.14	13.59	1.87	1.03	0.593
0.10	10.90	15.92	1.87	1.20	0.639
0.05	9.50	13.23	1.52	1.09	0.705
0.01	11.11	14.16	1.73	1.21	0.710
0.005	9.62	13.76	1.53	1.03	0.673
0.0	11.63	16.11	1.80	1.36	0.758

### 5. Interaction of *Glomus intraradices* and *Meloidogyne arenaria* on growth of peanut cultivar 'Florunner'.

Plant parasitic nematodes and vesicular-arbuscular mycorrhizal fungi are indigenous to most soils where peanuts are grown and can occur together in the root and rhizosphere of the same plant. *Meloidogyne arenaria* (Neal) Chitwood is the nematode species associated with peanuts that causes root knot. *M. arenaria* and other root-knot nematodes disrupt root function and nutrient absorption, reduce the number of feeder roots and suppress plant growth and yield. VAMF are obligately symbiotic fungi that colonize cortical cells of young roots and enhance, through an extensive network of extramatrical hyphae, the plants ability to absorb phosphorus (P), micronutrients and water.

Observations on the association of VAMF with plant parasitic nematodes and beneficial effect of mycorrhizal symbiosis on plant growth led to investigations into the potential of VAMF for limiting growth reduction and yield losses due to nematodes. This study was designed to determine the effect of *Glomus intraradices* on growth of peanut seedlings simultaneously inoculated with *M. arenaria* and the effect of *G. intraradices* on penetration of peanut roots by *M. arenaria* and post-infection development of *M. arenaria* within the root system.

The experimental design was a randomized complete block design and was conducted to evaluate the effect of *G. intraradices* on host susceptibility to nematode penetration and colonization and on host tolerance to parasitism by *M. arenaria*. Treatments were as follows: (1) inoculation with *G. intraradices* and *M. arenaria* (2) inoculation with *G. intraradices*; (3) inoculation with *M. arenaria*; and (4) control—no *G. intraradices*, no *M. arenaria*.

All peanut seedlings were grown in 25.4 cm diameter pots containing pasteurized river sand. Immediately prior to planting, appropriate pots received equivalent amounts of mycorrhizal inoculum (450 cc of *G. intraradices*) which consisted of pasteurized soil mixed with infected root segments and chlamydo spores. The inoculum was placed in a 2 cm deep layer approximately 1.25 cm below the seeds. Non-mycorrhizal treatments each received 450 cc of pasteurized soil only. Three seeds were sown per pot and seedlings were thinned to one seedling per pot prior to nematode introduction.

Each pot was top-dressed with 20 g of Osmocote 19-6-12 controlled release fertilizer immediately after planting. In addition all pots received one application of Peter's STEM micronutrient mix at the recommended level.

*Meloidogyne arenaria* was propagated on tomato plants grown in the greenhouse. Eggs were extracted with a 0.5% NaOCl solution, quantified and separated into allotments of approximately 10,000. The inoculum, consisting of 10,000 eggs suspended in 12 ml of tap water, was applied to appropriate plants 10 days after planting. Three ml aliquots, each containing 250 eggs were pipetted into each of four holes 4 cm deep positioned around each plant. After nematode inoculation each pot in the experiment received an equal amount of deionized water every 3 days for the duration of the experiment.

Plants receiving each treatment were harvested 10, 14, and 54 days after inoculation with nematode eggs. At each harvest plant heights, number of stems, number of leaves, and root and shoot fresh weights were recorded. Leaves and stems were dried at 80°C for 72 hours in a Precision Scientific model 645 drying oven prior to recording dry weights. Leaf areas were determined at the second and third harvest using a Li-Cor model LI-3000 portable area meter. Five staining and clearing procedures were attempted to visualize both nematodes and mycorrhizal fungi. These consisted of (1) clearing with KOH and staining; (2) clearing with hydrogen peroxide and staining; (3) staining with acid fuchsin; (4) staining with trypan blue; and (5) staining with chlorazol black E. Several heating procedures were evaluated (i.e. microwave, hot plate and autoclave). In the selected clearing and staining procedure, 1 cm segments were cleared for 1 h in 30% hydrogen peroxide, then stained with lactophenol blue using a modified version of the technique described by Hayman and Phillips. After staining, of the test roots, evaluation of the whole root systems was made for the presence of hyphae, vesicles, arbuscules, and chlamydo spores of *G. intraradices*. The colonized root length, total root length and percent of root colonization were determined. The percent of roots penetrated by *M. arenaria* larvae, the number of larvae that penetrate each root system and the developmental stage of the nematodes present were recorded.

Plant growth. Neither inoculation of peanut roots with *M. arenaria* nor *G. intraradices* alone nor dual inoculation influenced the number of stems, leaves or plant heights 10 and 24 days after nematode introduction; however, seedlings inoculated with *M. arenaria* demonstrated depressed shoot fresh weights compared to control plants or those inoculated with *G. intraradices* alone (Tables 11 and 12). Leaf area measurements recorded 14 days after nematode introduction also indicated this trend. At 10 and 24 days after nematode introduction dual inoculation with *G. intraradices* and *M. arenaria* resulted in shoot fresh weights, shoot dry weights and leaf areas very similar to those obtained when seedlings were inoculated with *M. arenaria* alone. At this time *G. intraradices* had little, if any effect of growth suppression due to root colonization by *M. arenaria*.

At both 10 and 24 days after nematode inoculation, seedlings inoculated with *G. intraradices* alone possessed greater shoot fresh and dry weights than control plants, indicating a mycorrhizal effect on growth was beginning to occur. Ten days after nematode introduction seedlings colonized by *G. intraradices* possessed greater root fresh weights than did any other treatments. By 24 days, however, root fresh weights were greater in *M. arenaria* infected plants than in control plants, plants inoculated with *G. intraradices* alone or dually inoculated plants. This trend of higher root weight and decreased shoot weight in plants inoculated with *M. arenaria* was reflected in higher root-shoot ratios (Table 12).

Fifty four days after nematode inoculation plants inoculated with *G. intraradices* and those inoculated with both *G. intraradices* and *M. arenaria* had significantly greater plant heights, numbers of stems and leaves, shoot dry weights and leaf areas than control plants or plants inoculated with *M. arenaria* alone. Greater than threefold differences were observed in leaf areas, number of leaves and shoot dry weights (Table 13). Growth of control plants differed only slightly from that of plants inoculated with *M. arenaria*, indicating that infection by these root-knot nematodes did not result in any significant growth depression in host plants. Mycorrhizal colonization resulted in substantial stimulation of plant growth and nullified any inhibitory effect of *M. arenaria*.

Table 11. Effect of *Glomus intraradices* and *Meloidogyne arenaria* on growth of peanut seedlings (cv. 'Florunner') 10 days after introduction of nematodes.

Treatment	Plant height cm	Number of Stems	Number of Leaves	Root fresh Weight g.	Shoot fresh Weight g.	Root/Shoot Ratio	Leaf dry Weight mg.	Stem dry Weight mg.
<i>Glomus intraradices</i> & <i>Meloidogyne arenaria</i>	11.57	3.3	11.3	3.82	6.90	0.609	766.60	320.77
<i>Glomus intraradices</i>	15.90	4.0	16.7	5.29	11.32	0.471	978.10	383.90
<i>Meloidogyne arenaria</i>	12.30	3.0	12.0	4.08	6.88	0.609	697.37	320.97
Control	14.67	3.7	14.0	4.77	8.79	0.556	830.70	376.40

All values listed are means of three replications.

**Table 12. Effect of *Glomus intraradices* and *Meloidogyne arenaria* on growth of peanut seedlings (cv. 'Florunner') 24 days after nematode introduction.**

Treatment	Plant height cm	Number of Stems	Number of Leaves	Root fresh Weight g.	Shoot fresh Weight g.	Root/Shoot Ratio	Leaf dry Weight mg.	Stem dry Weight mg.	Leaf Area cm <sup>2</sup>
<i>Glomus intraradices</i> & <i>Meloidogyne arenaria</i>	18.60	5.0	26.33	5.14	12.75	0.404	1603.33	716.67	399.42
<i>Glomus intraradices</i>	22.33	3.7	25.67	5.16	16.60	0.309	2330.00	963.33	462.18
<i>Meloidogyne arenaria</i>	16.37	4.7	23.33	6.61	11.33	0.598	1590.00	736.67	320.23
Control	18.83	4.3	23.33	5.33	13.03	0.401	1670.00	893.33	341.87

All values listed are means of three replications.

**Table 13. Effect of *Glomus intraradices* and *Meloidogyne arenaria* on growth of peanut seedlings (cv. 'Florunner') 54 days after nematode introduction.**

Treatment	Plant height cm	Number of Stems	Number of Leaves	Root fresh Weight g.	Leaf Dry Weight g.	Stem dry Weight g.	Leaf Area cm <sup>2</sup>
<i>Glomus intraradices</i> & <i>Meloidogyne arenaria</i>	28.23	9.3	82.00	8.47	7.22	4.50	1233.32
<i>Glomus intraradices</i>	30.00	10.3	84.33	7.88	7.71	5.21	1200.36
<i>Meloidogyne arenaria</i>	19.70	4.0	30.33	6.30	2.16	1.69	364.15
Control	21.83	3.67	31.33	6.30	2.32	1.73	352.84

All values listed are means of three replications.

Experimental results indicated only a slight mycorrhizal effect on growth 10 days after nematode introduction. Nematode inoculation occurred, on the average, 3 days after seedling emergence. Mycorrhizal colonization was in the initial stage at this point in time. Mycorrhizal growth enhancement was more pronounced, although still not significant, 24 days after nematode introduction. A substantial mycorrhizal effect was not documented until 54 days after nematode introduction. A lag period between initial mycorrhizal colonization and initiation of physiological changes altering plant growth has been commonly observed.

The sequence in which plants are inoculated with nematodes relative to the time of VAMF inoculation may affect the interaction between these two organisms. When VAMF colonize roots simultaneously with plant parasitic nematodes, growth responses caused by VAMF are often not observed for 4-6 weeks after fungal inoculation. Simultaneous inoculation resulted in no significant differences in shoot weights between dually inoculated seedlings or those inoculated with *M. arenaria* alone. With *M. arenaria* inoculation 28 days after *G. intraradices* inoculation, shoot growth was enhanced after only 7 days.

VAMF have been shown to enhance the uptake of Ca, Cu, Mn, S, and Zn in addition to P and increase water uptake. Nematode damaged plants frequently demonstrate impaired water conductance through roots and deficiencies of B, N, Fe, P, Mg, and Zn. VAMF may increase host tolerance by increasing uptake of key nutrients that would be deficient in a non-mycorrhizal nematode infected plant.

These results support the hypothesis that VAMF induced growth enhancement of peanut seedlings and increased tolerance to *M. arenaria* was due to enhanced uptake of key nutrients. Growth of *M. arenaria* inoculated plants was not significantly different from control plants indicating lack of essential nutrients was most likely the cause of suppressed growth rather than physical damage or physiological changes within roots induced by the nematodes themselves. Further work is needed on this beneficial effect of VAMF.

The interaction of *G. intraradices* and *M. arenaria* within peanut roots will become much clearer when growth data can be correlated with penetration and post infection development results. These data are being compiled and will be presented next year.

#### 6. Effect of field inoculation with *Glomus deserticola* on growth of peanut cv. 'Florunner'.

A field experiment was conducted at the Texas A&M University Yoakum Experiment and Research Center to determine the effect of three inoculation levels of *Glomus deserticola* on the growth of peanut cultivar 'Florunner'.

##### Experimental Design

1. Randomized complete block design
2. 4 replication
3. Single row plots—one row = 6.1 meters
4. 4 Treatments
  - Level 1 = 700 ml inoculum (35ml (14,000 spores) inoculum/ft + 2300 ml river sand
  - Level 2 = 1400 ml inoculum (70 ml (28,000 spores) inoculum/ft + 1600 ml river sand
  - Level 3 = 2100 ml inoculum (105 ml (42,000 spores) inoculum/ft) + 900 ml river sand
  - Control = 3000 ml river sand
5. Inoculum—*Glomus deserticola* (400 spores/ml) from Native Plants Incorporated, Salt Lake City, Utah.
6. Inoculum placed at 10 cm. below surface and seed placed directly on top.
7. 7.06 grams Nitragin "PG" *Bradyrhizobium* inoculum per row (all treatments).
8. Florunner cultivar.
9. Mechanically planted June 30, 1987 using a V-belt seeder.
10. Harvested November 13, 1987.

##### Results

At 40 days, the top weights of plants inoculated with *G. deserticola* that received the two higher levels of inoculum were significantly greater than control top weights. See Fig. 1 and Table 14. The lower inoculum level resulted in plants having top weights similar to those of the controls. The dry weights of level two plants were significantly greater than the dry weights of control plants (Fig. 2 and Table 14). No differences were found between root system weights (Fig. 3). Yield data are presented in Table 15. The two lower inoculum levels applied to plants resulted in production of plants having greater yields than those of the control plants. See Fig. 4.

**Table 14. Effect of *Glomus deserticola* on growth of Florunner peanuts under four inoculum level regimes. 40 days.**

Rep	Fresh Shoot Weight	Dry Shoot Weight	Root Weight
Level 1 (14,000 spores/ft)			
1	161.3	38.7	1.9
2	155.9	37.3	1.6
3	143.9	32.8	1.9
4	148.8	34.0	1.8
Level 2 (28,000 spores/ft)			
1	168.4	39.7	1.8
2	168.6	37.6	1.6
3	201.9	41.8	1.9
4	199.6	40.5	2.0
Level 3 (42,000 spores/ft)			
1	179.5	35.3	1.8
2	209.4	47.4	1.9
3	192.3	40.6	2.1
4	225.8	53.3	2.5
Control (no mycorrhizal inoculum)			
1	133.3	35.3	2.0
2	175.0	37.8	1.9
3	131.7	34.5	1.4
4	123.4	30.1	1.7

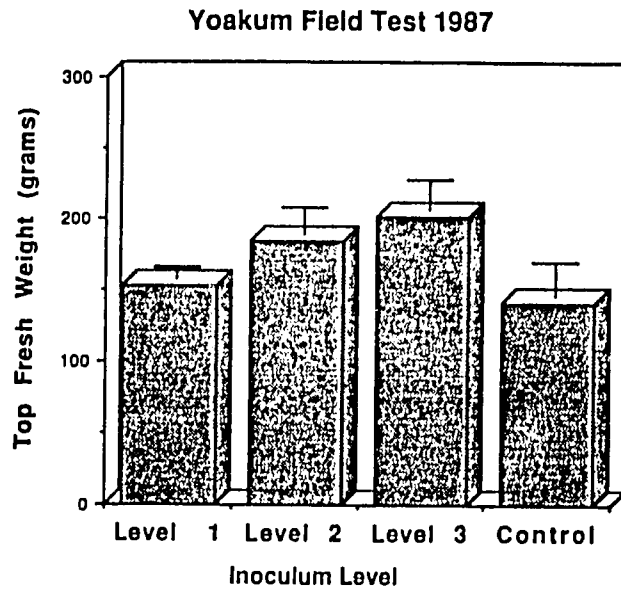


Figure 1. Effect of *Glomus deserticola* inoculum level on fresh top weights of Florunner peanuts at 40 days. Field inoculation with no fumigation. Level 1 = 14,000 spores/foot, Level 2 = 28,000 spores/foot, Level 3 = 42,000 spores/foot and Control = no mycorrhizal inoculum.

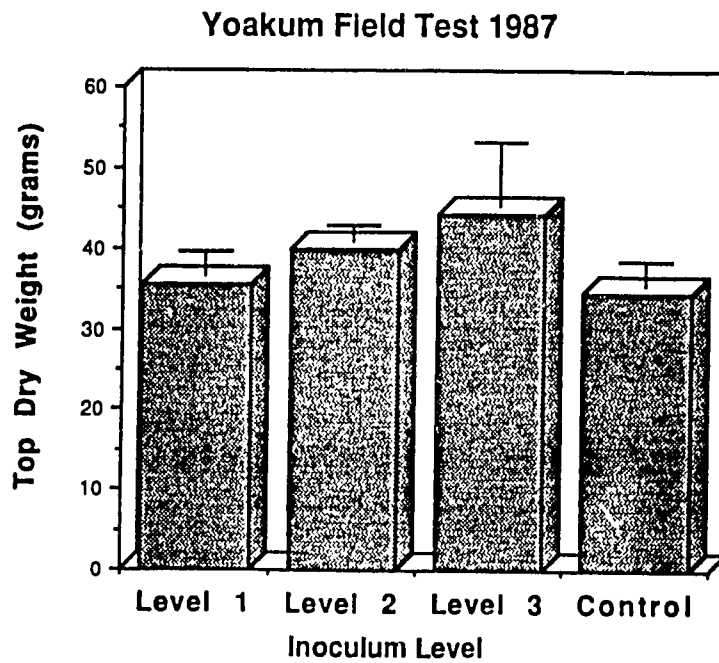


Figure 2. Effect of *Glomus deserticola* inoculum level on dry top weights of Florunner peanuts at 40 days. Field inoculation with no fumigation. Level 1 = 14,000 spores/foot, Level 2 = 28,000 spores/foot, Level 3 = 42,000 spores/foot and Control = no mycorrhizal inoculum.

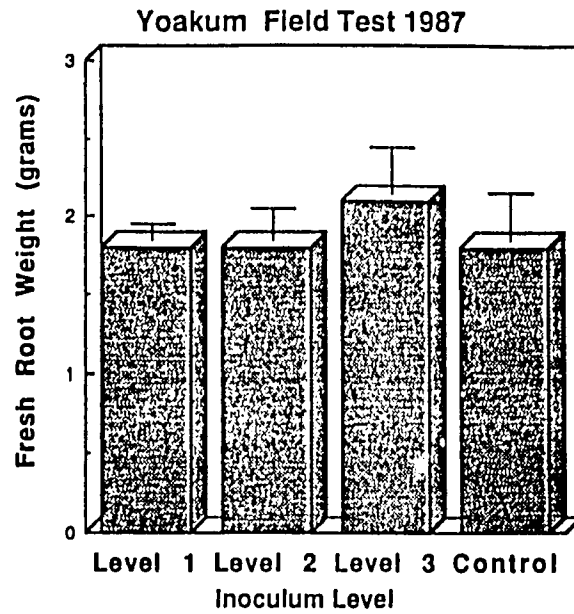


Figure 3. Effect of *Glomus deserticola* inoculum level on root weights of Florunner peanuts at 40 days. Field inoculation with no fumigation. Level 1 = 14,000 spores/foot, Level 2 = 28,000 spores/foot, Level 3 = 42,000 spores/foot and Control = no mycorrhizal inoculum.

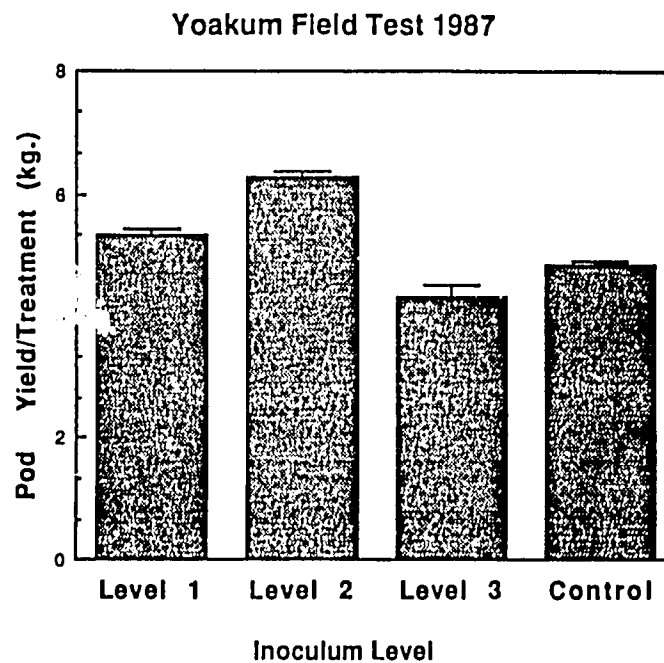


Figure 4. Effect of *Glomus deserticola* inoculum level on yield of Florunner peanuts at harvest. Field inoculation with no fumigation. Level 1 = 14,000 spores/foot, Level 2 = 28,000 spores/foot, Level 3 = 42,000 spores/foot and Control = no mycorrhizal inoculum.



Table 15. Effect of field inoculation with *Glomus deserticola* on Florunner seed quality and yield.

REPS	SMK	SS	OK	DAMAGED	HULLS	YIELD Kg/Rep
Level 1 (14,000 spores/ft)						
1	141	11	9	3	36	5.39
2	142	4	12	2	40	5.50
3	137	8	12	3	40	4.95
4	137	11	11	0	41	5.50
Level 2 (28,000 spores/ft)						
1	136	13	8	3	40	6.38
2	150	3	10	1	36	6.05
3	133	14	13	0	39	6.27
4	143	6	12	2	36	6.49
Level 3 (42,000 spores/ft)						
1	144	6	10	2	37	3.74
2	137	13	12	1	36	4.29
3	130	8	20	0	41	3.85
4	143	11	8	0	38	4.95
Control (no mycorrhizal inoculum)						
1	136	12	8	2	43	4.62
2	135	9	5	2	28	4.95
3	134	8	4	6	38	4.84
4	139	5	6	1	38	4.95

## PHILIPPINES AND THAILAND

Funding to Philippines and Thailand ended with the 1986-87 year; therefore, there are no research reports from the host countries.

## TRAINING OUTPUTS

July 20-24, 1987—Dr. Philippe Sankara of Ouagadougou, Africa

## TRAVEL

July 1987—Trip to Senegal, West Africa for the collection of mycorrhiza germplasm. Funded by Texas A&M University Office of International Agricultural Programs

Dr. Joe Morton's lab at West Virginia University, Morgantown, West Virginia. Identification of VA mycorrhizal fungi.

USDA lab Beckley, West Virginia—Dr. Sara Wright—monoclonal antibodies—arrangements for Randy Garber to study there.

## PRESENTATIONS

Neck, J. S., R. K. Garber and R. A. Taber. 1987. Effect of phosphorus on growth and mycorrhizal response of *Arachis hypogaea*. 7th North American Conference on Mycorrhizae. Gainesville Florida.

Garber, R. A., J. S. Neck and R. A. Taber. 1987. Influence of *Glomus etunicatum* and phosphorus on nitrogen fixation in peanuts. 7th North American Conference on Mycorrhizae. Gainesville Florida.

Ilag, Lina, Elsa A. Parot, and R. A. Taber. 1987. Effect of varying *Glomus versiforme* inocula on peanut growth. 7th North American Conference on Mycorrhizae. Gainesville Florida.

Taber, R. A., R. E. Pettit, J. S. Neck, O. D. Smith, D. H. Smith, and W. L. Harman. 1987. Responses of five peanut cultivars to field inoculation with four mycorrhizal fungi. Best Plant Pathology paper at American Peanut Research and Education Society—Orlando July 13-17, 1987.

Neck, J. S. and R. A. Taber. 1988. Growth stimulation of peanut in response to mycorrhizal colonization American Peanut Research and Education Society. Tulsa, Oklahoma.

## PUBLICATIONS

Rajapakse, S., R. A. Taber and J. C. Miller. 1987. Cultivar variability for VA mycorrhizae symbiosis in field grown cowpea under three soil treatments. *New Phytologist*.

Taber, R. A., R. E. Pettit, J. S. Neck, O. D. Smith, D. H. Smith, and W. L. Harman. Responses of five peanut cultivars to field inoculation with four mycorrhizal fungi. (In review).

## PLANS FOR 1988-89

J. Stephen Neck and Randall Garber will complete their mycorrhizal research for their Ph.D. dissertations. Mr. Randy Garber will spend time at the USDA Beckley, West Virginia laboratory to develop monoclonal antibodies against *Glomus* sp. Monoclonal antibodies will be used in his research to identify species.

AAMU/FT/CARDI  
**Peanut Utilization in Food Systems of  
Developing Countries**

Alabama A & M University  
Caribbean Agricultural Research and Development Institute  
University of the West Indies, St. Augustine Campus, Trinidad  
Bharat Singh, Principal Investigator, AAMU

## **INTRODUCTION**

Scientists from the Peanut CRSP (AAMU) and the CARDI completed a survey on consumption and post-harvest handling of peanuts in Trinidad, St. Vincent and Jamaica in May 1984 to provide the basis for further research on peanuts in the region. The data from the survey indicated that one of the major problems is the quality of peanuts for processing particularly for peanut butter. There are also problems of mold contamination during storage and handling. Additional storage and handling problems include drying and curing of peanuts and separation of the testa. Information on the quality of peanut produced in Jamaica is just not available. Locally grown peanuts present problems to processors of peanut butter especially oil separation, poor texture and undesirable color. This report presents data on studies completed on varieties/lines of peanuts grown in Jamaica and a study on determination of maturity indices for peanuts grown in eastern Caribbean regions.

## **MAJOR ACCOMPLISHMENTS:**

- 1) Proximate compositions, fatty acids and amino acids were determined on cultivars grown at Lawrencefield, Jamaica in 1985 and 1986.
- 2) Physical characteristics and sensory evaluation data on pods have been determined to provide basis for establishing maturity indices for cultivar NC2 grown in the eastern Caribbean regions.

## **REALIZED AND EXPECTED IMPACT OF PROJECT**

### **HOST COUNTRY**

1. The project has established linkages with CARDI, University of the West Indies, and Food Technology Institute. It is expected that these linkages will result in long-term relationships between the collaborating institutions even after the project is ended.

2. Data from the consumption and postharvest surveys have already provided information that has helped to define appropriate areas for research on peanuts in Caribbean nations.

3. It is expected that the research on quality evaluation, maturity, storage, preservation, and preparation for consumption will lead to improved means of storage and innovative means of processing peanut in various Caribbean countries. These rural, small-farm, Caribbean populations may have increased and prolonged opportunities to benefit from increased peanut production and consumption.

4. The project specifically will enhance the capabilities of CARDI, the University of the West Indies, and the Food Technology Institute, enabling each to do research on peanut, peanut products, and other food products.

### **UNITED STATES**

1. The information on quality aspects of cultivars grown in the Caribbean region will be useful to scientists in the U.S.A. in determining performance at locations varying in soil types and environmental condition.

2. The project has provided an opportunity for Alabama A & M University to acquire the regional experience in conducting research related to solving food problems in the Caribbean region.

## GOALS

The major goal of this research project is to develop the means for greater utilization of peanut for food by developing new foods or improving existing ones with peanuts as an ingredient.

## OBJECTIVES

The overall objectives are:

- A. To describe and to understand any variations in environment, socioeconomics, and food technologies as they constrain the preservation and utilization of peanut supplies.
- B. To analyze the current and potential dietary role of existing peanut products.
- C. To assess the sensory, nutritional, microbiological, and toxicological quality parameters of peanut products.
- D. To incorporate indigenous peanuts and peanut products into solid and/or beverage food systems for local consumption.
- E. To prepare and to present peanut fortified foods in an effort to determine acceptability and nutritional values of such products.
- F. To assure safety of the products with particular reference to mycotoxins in raw and finished products.

## ORGANIZATION

### Alabama A&M University

Dr. B. Singh, Food Scientist, Coordinator, Department of Food Science, Normal, AL

Dr. B. Onuma Okezie, Food Scientist, Cooperator, Department of Food Science, Normal, AL

Dr. John C. Anderson, Food Scientist, Cooperator, Department of Food Science, Normal, AL

Dr. G. C. Wheelock, Rural Sociologist, Cooperator, Department of Agribusiness, Normal, AL

### University of the West Indies, St. Augustine Campus, Trinidad

Dr. George Sammy, Food Scientist (deceased)

Ms. Margaret Hinds, Graduate Research Assistant

### Graduate Students and Research for Theses

Mr. E. Miller, Studies on postharvest handling and storage of peanuts with special reference to Jamaica (completed M.S.).

Miss Margaret Hinds (started 1985), Postharvest and compositional studies on peanuts grown in St. Vincent and Antigua.

## SPECIFIC OBJECTIVES:

Research plans were made on the basis of base-line data obtained through the surveys conducted in 1984-1985. These objectives were:

1. To conduct research on postharvest handling of peanuts including (a) quality evaluation in collaboration with plant scientists in Caribbean countries and the University of Georgia. (b) Improvement of curing and storage methods.
2. To conduct research on modification of peanut processing: (a) roasted peanuts. (b) Peanut butter and other products.
3. To monitor aflatoxins and to demonstrate their decontamination in peanut products: (a) Monitoring of aflatoxins in peanuts produced in various Caribbean countries. (b) Development of method(s) to decontaminate peanuts.
4. To conduct research on nutritional inhibitors or factors which constrain peanut utilization, especially inhibitors.

5. To train Caribbean students and scientists in order to realize long-term effects of the Peanut CRSP on institution building in the region.

#### ACCOMPLISHMENTS IN DETAIL

Research was conducted to accomplish proposed objectives at Alabama A&M University and the University of the West Indies.

#### DETERMINING MATURITY INDICES FOR PEANUT (*Arachis hypogaea* L.), cultivar NC2. (Margaret J. Hinds, George M. Sammy & Bharat Singh)

The aim of this research is to establish maturity indices for the NC2 peanut cultivar from composition and physical characteristics of composite pods, and to confirm them using Sensory evaluation of the seeds.

The NC2 variety was introduced into St Vincent about eight years ago. Agronomic trials by C.A.R.E.I. with this variety seem to show it very suitable to those climatic and soil conditions. However the nutrient composition of this cultivar grown in St. Vincent has not yet been investigated. Furthermore, a time factor (viz. 120 days after planting) is used to determine reaping time. Thus, in many instances the marketed pods are of poor quality—either too young or too old and moldy. Therefore, there is a need for more reliable maturity indices to be devised. In this study it is hypothesized that composition and physical characteristics of composite pods sampled periodically during one season should be similar to those from other seasons although the rate of attainment of these characteristics may be different. Consequently, an investigation is being conducted to determine if optimum reaping time could be established from any individual characteristic or combination of characteristics of composite samples. In this context, a composite sample is the mixture of pods (of all physiological stages) obtained from a group of 20 or more plants reaped from one area (block) of the plot on the same day. Tests using composite samples would be objective and should be fairly accurate since there would be no need to divide samples subjectively into their different physiological classes.

The following physical, biochemical and sensory tests are being carried out on mature, nearly mature, intermediate and composite pods and seeds from plants of different ages (days after planting): (a) pod length, (b) pod diameter, (c) %-nut-volume of green pods, (d) %-nut-weight of green pods, (e) seed/shell ratio of green pods, (f) dried pod weight (g) dried seed weight, (h) seed/hull ratio of dried pods, (i) %-dry matter in seeds, (j) %-ash in seeds, (k) %-crude lipid in seeds, (l) %-crude protein in seeds- (m) lipid/protein ratio in seeds, (n) total amino acid concentration in seeds, (o) T/AT amino acid ratio in seeds, i.e., amino acids responsible for typical flavor/amino acids responsible for atypical flavour, (p) total fatty acid concentration in seeds, (q) oleic acid/linoleic acid ratio in seeds, (r) sensory characteristics of paste from roasted seeds of air-dried pods, and (s) texture of roasted seeds from air-dried pods.

For each season a plot was divided into blocks and samples were collected following a Random Complete Block design; plants being removed from three locations within the plot on each day of sampling (i.e., 3 replicates per treatment). Samples were collected over three seasons from the two main soil types on which peanuts are usually grown as presented in Table 1.

**Table 1: Schedule for Sample Collection**

Season++ Days after planting	I		II Soil type+		III	
	v-s	v-c	v-s	v-c	v-s	v-c
99		*				
106	*	*	*	*		
113	*	*	*	*	*	*
120	*	*	*	*	*	*
123					*	*
127	*	*	*	*	*	*
130					*	*
134	*		*	*	*	*
137					*	*
141					*	*

+ Soil types: v-s, volcanic-sandy; v-c, volcanic-clayey

++ Seasons: I, Jan. - Mar. 1986; II, Aug. - Nov. 1986;

III, Sep. - Oct. 1987

Pods (i.e., kernels intact in their hulls) for physical tests and composition analysis were kept frozen while those for sensory evaluation were air-dried to a moisture content of approximately 8%. Pods were classified on the basis of laboratory observations using the method of Pattee et al (1974) into:

- (a) Mature pods = Stage 13
- (b) Nearly mature pods = Stages 11 & 13
- (c) Intermediate pods = Stages 9 & 10

## PHYSICAL TESTS

Notes on methods and apparatus are as follows:

length & diameter of pods with their 'beaks' facing upwards (stainless steel micrometer);

%-nut-volume of green pods = volume of seeds/volume of pods (AOAC, 1980: 227.001) for pods with 100ml measuring cylinder for seeds (i.e., kernels without seedcoats); %-nut-weight of green pods = wt of seeds/wt of pods;

Seed/shell ratio for green pods = wt of seeds/wt of shells.

To obtain 'dried weights' the individual pod components were dried to constant weight in an air-oven @ 150°C for 5 - 6 hours;

Dried pod weight = (wt of seeds + testas + shell)/no. of pods;

Dried seed weight = total wt of seeds/no. of seeds

Dried seed/hull ratio = wt of seeds/wt of hulls.

(A triple beam balance was employed in weighing.)

Sensory evaluation was performed to determine if the recommendations for harvesting from the composition and physical tests correlate positively with the samples of highest consumer-acceptability. Mature, nearly mature, intermediate and composite seeds from all treatments (season III) were individually roasted and ground into paste (paste used to ensure that sub-samples from composite seeds were uniform). An Incomplete Block design was used for assigning sub-samples to a semi-trained panel. The paste was evaluated for color, aroma and taste on a hedonic scale. Roasted seeds from mature, nearly mature and intermediate pods were also evaluated for aroma, appearance, color, crunchiness, mouthfeel and taste using a similar statistical design.

## STATISTICAL ANALYSIS

Analysis of variance combined with Duncan's Multiple Range Test (where necessary), and Linear Regression Analysis were carried out using the SAS Routine Library on an IBM computer.

Physical tests on all samples collected from the 3 seasons plus sensory evaluation of season III nuts have been completed. However, the results and discussion presented are mainly from season I samples since the other data are still being analyzed.

### PERCENTAGE OF MATURE PODS

**Table 2: Percentage Mature Pods from Volcanic-Clayey Soil (Season I)**

DAP*	Location 1	Location 2	Mean
99	0	0	0
106	2	2	2
113	12	11	12
120	45	45.5	45
127	76	76	76

\*DAP = Days after planting

A count of numbers of mature to immature pods was performed on total pod yield from 20 plants sampled from two different locations within the plot on five different days of reaping. The results (Table 2) indicated that the percentage of mature pods from plants of similar ages (days after planting) were comparable; and that the percentage of mature pods increased significantly with the age of the plants. Therefore, at optimum reaping time the majority of plants should contain some consistently large proportion of mature pods to immature pods.

### COMPARISON OF PHYSICAL CHARACTERISTICS OF MATURE, NEARLY MATURE & INTERMEDIATE PODS:

Tests were performed separately on mature and developing pods so that eventually some standards for mature pods might be obtained. The data should provide guidelines for monitoring changes in composite pods. Generally, the results suggested that after the intermediate stage shells grow at faster rates than kernels and attain their maximum size. Then, the kernels swell out and the mature pod is obtained.

**Table 3: Characteristics of Mature, Nearly Mature & Intermediate Pods (Season I)**

Characteristic	Mature	Nearly	Intermediate Mature
Pod length (cm)	3.51	3.37	3.29
Pod diameter (cm)	1.45	1.39	1.39
%-nut-volume	41	39	40
%-nut-weight	77.2	68.9	70.0
Seed/shell (green) ratio	3.45	3.70	3.19
Dried pod wt (g)	1.93	1.72	1.71
Dried seed wt (g)	0.76	0.69	0.69
Seed/hull (dried) ratio	3.10	2.87	2.88

### MEAN POD LENGTHS

Mean pod lengths of composite samples from v-s soil peaked at 3.73 cm and then decreased, while those from v-c soil reached a maximum of 3.28 on the last sampling day (Table 4). Mean lengths of pods from v-s soil plants were generally larger than those from v-c soil plants of similar ages.

**Table 4: Mean Lengths (CM) of Green Composite Pods (Season I)**

DAP	Volcanic-sandy Soil			Volcanic-clayey Soil			
	Loc 1*	Loc 2*	Mean $\pm$ SD	Loc 1**	Loc 2**	Loc 3**	Mean $\pm$ SD
99				3.17	3.02	3.03	3.07 $\pm$ .08
106	3.25	3.46	3.36 $\pm$ .15	3.12	3.15	3.03	3.10 $\pm$ .06
113	3.64	3.70	3.67 $\pm$ .04	3.18	3.11	3.14	3.14 $\pm$ .04
120	3.61	3.85	3.73 $\pm$ .17	3.28	3.14	3.15	3.19 $\pm$ .18
127	3.77	3.56	3.67 $\pm$ .15	3.26	3.28	3.31	3.28 $\pm$ .03
134	3.93	3.43	3.68 $\pm$ .35				

\* Location values are means of 50 determinations per each v-s location and sampling date.

\*\* Location values are means of 75 determinations per each v-c location and sampling date.

The difference in rainfall between the two plots during pod development (weekly rainfall averaged 2.97 cm & 1.16 cm for v-s and v-c soils, respectively) probably contributed to the v-s soil yielding relatively longer pods. Thus it seems that degree of maturity of a pod cannot be reliably estimated from its length.

Statistical analysis indicated that mean pod lengths of composite samples from v-c soil were significantly different among treatments (1% level) as well as between replicates (5% level) while those from v-s soil were neither significantly different among treatments nor between replicates. Although the mean pod lengths of the v-c samples increased continually during the sampling period, linear regression analysis on the 12 data points from the first 4 digging days did not seem suitable for predicting mean pod lengths on subsequent days (e.g., pod length at 127 DAP was predicted to be 3.2 cm). From the mean pod length values one might assume that the greatest proportion of mature pods were probably present at 120 and 127 DAP for v-s and v-c samples, respectively. However, the use of mean pod lengths of composite samples does not seem reliable enough for estimating optimum reaping date.

#### POD DIAMETER

Table 5 implies that the mean diameter of the composite pods from v-s soil plants were larger than those from the v-c soil plants of similar ages. The difference in the rainfall on the two plots during pod development could have contributed to this trend.

**Table 5: Mean Diameters (CM) of Green Composite Pods (Season I)**

DAP	Volcanic-sandy Soil			Volcanic-clayey Soil			
	Loc 1*	Loc 2*	Mean $\pm$ SD	Loc 1**	Loc 2**	Loc 3**	Mean $\pm$ SE
99				1.40	1.36	1.35	1.37 $\pm$ .03
106	1.44	1.48	1.46 $\pm$ .03	1.36	1.35	1.32	1.34 $\pm$ .02
113	1.50	1.48	1.49 $\pm$ .01	1.36	1.35	1.35	1.35 $\pm$ .01
120	1.46	1.51	1.49 $\pm$ .04	1.38	1.37	1.37	1.37 $\pm$ .01
127	1.52	1.55	1.54 $\pm$ .02	1.37	1.41	1.39	1.39 $\pm$ .02
134	1.41	1.43	1.42 $\pm$ .01				

\* Location values are means of 50 determinations per each v-s location and sampling date.

\*\* Location values are means of 75 determinations per each v-c location and sampling date.

Although from the mean values it may appear that for both soil types the largest proportion of broad pods were present at 127 DAP, statistical analysis indicated that there was no significant difference among the treatments from each soil type. Linear regression analysis of the early data points did not facilitate predictions about the later sampling days. Thus, from season I it would appear that the mean diameter of composite pods should not be used by itself for determining optimum harvest date.



**%-NUT-VOLUME OF GREEN PODS (VOL. OF SEEDS/VOL. OF PODS)****Table 6: %-Nut-Volume of Green Composite Pods (Season I)**

DAP	Volcanic-sandy Soil			Volcanic-clayey Soil			
	Loc 1*	Loc 2*	Mean $\pm$ SD	Loc 1**	Loc 2**	Loc 3**	Mean $\pm$ SD
99				24	25	24	24.3 $\pm$ 0.6
106	21	19	20.0 $\pm$ 1.4	28	29	29	28.7 $\pm$ 0.6
113	27	24	25.5 $\pm$ 2.1	33	36	33	34.0 $\pm$ 1.7
120	32	34	33.0 $\pm$ 1.4	39	38	36	37.7 $\pm$ 1.5
127	33	30	31.5 $\pm$ 2.1	40	40	39	39.7 $\pm$ 0.6
134	35	36	35.5 $\pm$ 0.7				

\* Means of duplicate tests per v-s location with 35 pods per test.

\*\* Means of triplicate tests per v-c location with 35 pods per test.

Observations made during this experiment have suggested that shells attain their maximum size before the corresponding kernels; and that during the final stage of pod development the kernels swell to maximum size while the shell merely becomes drier and firmer. Thus, the ratio of volume of seeds to pods was investigated as a possible quick and objective method to estimate actual nut content. It was thought that as this is a ratio it would not be as greatly influenced by environmental factors as pod length and diameter seem to be. Also (for each variety) a mature pod, irrespective of size, should contain a minimum percentage volume of kernel/seed. For both soil types the %-nut-volume of composite pods increased with age of plants, and the maximum values (35.5 for v-s and 39.7 for v-c) were obtained on the last day of sampling (Table 6). The value obtained for v-c mature pods was 41% (Table 3). This suggests that the largest proportions of mature pods were present on or after 134 and 127 DAP for v-s and v-c soil samples, respectively. Although the overall dimensions of the pods grown on v-s soil were larger than those from v-c soil, it was observed that pods from v-c soil plants contained a greater %-nut-volume than the corresponding v-s soil ones. It has been suggested that limited rainfall during pod development assists in increasing the rate of maturity. Thus, it is possible that the heavier rainfall on the v-s soil (Table 11) prolonged pod development, and thus the kernel-filling phase in many pods was incomplete on the last sampling day. Moreover, statistical analysis indicated that the increases in %-nut-volume of the v-c soil pods were significant (1% level) up to the 4th sampling day before tending to level off. In the case of the v-s soil pods, the difference between the 4th and 5th sets of samples was still significant suggesting that optimum yield might have been obtained after 134 DAP.

**%-NUT-WEIGHT OF GREEN PODS (WT OF SEEDS/WT OF PODS)****Table 7: %-Nut-Weight of Green Composite Pods (Season I)**

DAP	Volcanic-sandy Soil			Volcanic-clayey Soil			
	Loc 1*	Loc 2*	Mean $\pm$ SD	Loc 1**	Loc 2**	Loc 3**	Mean $\pm$ SD
99				38	40	41	39.7 $\pm$ 1.5
106	49	36	42.5 $\pm$ 9.2	44	43	45	44.0 $\pm$ 1.0
113	56	55	55.5 $\pm$ 0.7	53	57	54	54.7 $\pm$ 2.1
120	63	56	59.5 $\pm$ 5.0	61	65	60	62.0 $\pm$ 2.7
127	59	54	56.5 $\pm$ 3.5	63	63	62	62.7 $\pm$ 0.6
134	69	64	66.5 $\pm$ 3.5				

\* Means of duplicate tests;

\*\* Means of triplicate tests.

Although fresh pod weight may be influenced by environmental factors (e.g., rainfall) the %-nut-weight of the green pods was measured to find out if this inexpensive test may be useful to the farmer. Mean %-nut-weight of green composite pods from v-c soil increased with age of plants and the values from the first 4 diggings were significantly different from each other, but then leveled off after 120 DAP (Table 7). On the other hand, fluctuations in mean %-nut-weight of composite samples from v-s soil occurred so that the differences between both treatments and replicates were significant although heaviest %-nut-weight was obtained at 134 DAP. Comparing the %-nut-weights with the corresponding rainfall (Table 11) it would appear that a decrease in total weekly rainfall on the v-s soil from 0.86 cm to 0.25 cm may have contributed to the decrease in %-nut-weight at 127 DAP, and the subsequent rise at 134 DAP when the rainfall was 0.99 cm. The results so far tend to suggest that mean %-nut-weights of composite pods at optimum reaping time may depend on environmental factors. However, no conclusions about the reliability of this test can be drawn as yet since the %-nut-weights of the v-s soil pods beyond 134 DAP were not obtained.

#### SEED/SHELL RATIO OF GREEN PODS

Table 8: Seed/Shell Ratio of Green Composite Pods (Season I)

DAP	Volcanic-sandy Soil			Volcanic-clayey Soil			
	Loc 1*	Loc 2*	Mean $\pm$ SD	Loc 1**	Loc 2**	Loc 3**	Mean $\pm$ SD
99				0.86	0.93	0.85	0.88 $\pm$ 0.04
106	1.32	0.68	1.00 $\pm$ 0.45	1.10	1.03	1.13	1.09 $\pm$ 0.05
113	2.11	1.79	1.90 $\pm$ 0.30	1.76	1.71	1.52	1.66 $\pm$ 0.13
120	3.15	1.62	$\pm$ 1.08	2.02	1.87	1.93	1.94 $\pm$ 0.08
127	1.81	1.50	1.66 $\pm$ 0.22	2.41	2.40	2.41	2.41 $\pm$ 0.01
134	2.73	2.11	2.42 $\pm$ 0.44				

\* Single tests on 35 pods;

\*\* Means of triplicate tests on 35 pods.

Early in this study it was observed that the moisture content of testas from developing pods seemed to increase with dampness of soil; and in some cases the testas were relatively heavy from moisture surrounding the seed. It was therefore thought that the seed/shell ratio might be more suitable than the seed/hull ratio. Analysis of results (Table 8) showed that the seed/shell ratio of composite samples from v-c soil increased significantly between successive treatments up to the last day of sampling. Linear regression analysis of the first 12 data points predicted a seed/shell ratio of 2.33 at 127 DAP compared with the mean experimental value of 2.41. At 127 DAP the mean seed/shell ratio of the composite pods was 70% of that of the mature pods. Previous subjective classification of these composite pods showed that 76% were mature (Table 2). Although the maximum mean seed/shell ratio of composite pods from v-s soil was similar to that of v-c soil samples, no conclusions can be drawn due to significant differences between replicate samples and the fluctuations of the mean values. More tests are necessary to ascertain the usefulness of this method.

#### DRIED WEIGHTS OF PODS AND SEEDS

Results from Season I for plants grown on v-c soil indicate that the maximum values for composite dried pod and seed weights (Table 9) were 86% and 91%, respectively, of those of the mature samples (Table 3). By using linear regression on the 12 data points from the first 4 sampling days the values for dried pod and seed weights on the fifth sampling day were predicted to be 1.64 and 0.65, respectively. They are fairly close to the means of the actual experimental values (1.66 and 0.69, respectively) and, thus, suggest that linear regression on dried weights of composite samples may assist in predicting optimum reaping date.

**Table 9: Mean Dried Weights of Composite Pods and Seeds from Volcanic-Clayey Soil (Season I)**

DAP	Pod Weights*			Mean	Seed Weights*			Mean
	Loc1	Loc2	Loc3		Loc1	Loc2	Loc3	
99	1.24	1.25	1.23	1.24	0.52	0.51	0.49	0.51
106	1.15	1.22	1.20	1.19	0.46	0.53	0.54	0.51
113	1.45	1.43	1.39	1.42	0.61	0.64	0.57	0.61
120	1.60	1.53	1.51	1.55	0.63	0.55	0.62	0.60
127	1.65	1.66	1.67	1.66	0.70	0.68	0.70	0.69

\* Means of triplicate determinations

#### SEED/HULL RATIO OF DRIED PODS

The seed/hull ratio of the dried composite pods increased significantly (1 % level) with each sampling day and peaked at a mean value of 3.04 on the last day (Table 10). There was no statistically significant difference between values for replicate samples. The corresponding value obtained for mature pods was 3.1 indicating that at 127 DAP the composite pods had attained 98% of their potential maturity.

**Table 10: Seed/Hull Ratio of Dried Composite Pods from Volcanic-Clayey Soil (Season I)**

DAP	Loc 1*	Loc 2*	Loc 3*	Mean $\pm$ SD
99	1.80	1.82	1.79	1.80 $\pm$ 0.02
106	2.11	1.98	2.09	2.06 $\pm$ 0.07
113	2.54	2.65	2.50	2.56 $\pm$ 0.08
120	2.83	2.85	2.83	2.84 $\pm$ 0.01
127	3.06	3.00	3.05	3.04 $\pm$ 0.03

\* Means of triplicate tests

Previous studies using seed/hull ratio to estimate optimum reaping time for other varieties have indicated that although this procedure shows promise a reference value for each variety and location seems necessary. Experiments with NC2 in southern United States indicated a mean value of 3.4 for composite pods at optimum harvest time. From subsequent tests (in this study) there will be an attempt to establish a minimum seed/hull value corresponding to the presence of at least 75% mature pods.

**Table 11: Total Weekly Rainfall (CM) During Pod Development (Seasons I & II)**

Week	Season I		Season II	
	V-S	V-C	V-S	V-C
1	5.36	1.70	4.39	4.52
2	0.71	4.17	1.88	3.35
3	5.79	0.86	2.54	0.91
4	9.07	0.25	5.72	4.75
5	5.64	0.99	3.30	4.39
6	1.91	0.46	5.44	1.88
7	3.28	1.55	2.31	2.54
8	3.79	0.00	1.04	5.72
9	0.53	1.98	6.15	3.30
10	1.70	* 0.61	1.46	5.44
11	* 4.17	* 1.96	* 37.82	* 2.31
12	* 0.86	* 1.58	* 3.13	* 1.04
13	* 0.25	* 0.13	* 0.44	* 6.15
14	* 0.99	* 0.00	* 4.75	* 1.46
15	* 0.46	* 8.69	* 37.82	

\* Samples taken

#### CONCLUSIONS FROM PRELIMINARY EXPERIMENTS

- (i) The increase in numbers of mature pods among plants in the same season shows a fairly similar pattern.
- (ii) Composite pods and seeds from different farms tend to display some similar physical characteristics at various stages although their rates of attaining the same may differ depending on cultural, environmental and physiological factors.
- (iii) Some physical characteristics of mature pods and seeds tend to be statistically significantly different from those of immature ones.
- (iv) Some individual physical and composition characteristics of composite samples tend to indicate the relative proportion of mature to immature pods/seeds present. They are:
  - (a) %-nut-volume;
  - (b) %-nut-volume;
  - (c) Mean dried weights of pods and seeds
- (v) The following tests on composite samples do not seem reliable enough for predicting optimum reaping time:
  - (a) pod length
  - (b) pod diameter

#### PLAN OF RESEARCH FOR 1988 -1989

The following tests on samples from all 3 seasons have to be completed:

- (a) %-crude lipid in seeds
- (b) %-crude protein in seeds
- (c) Lipid/protein ratio in seeds
- (d) %-total amino acid content in seeds
- (e) T:AT amino acid ratio in seeds
- (f) %-total fatty acid content in seeds
- (g) Oleic/linoleic acid ratio in seeds

## A STUDY ON PROXIMATE COMPOSITION, AMINO ACIDS, AND FATTY ACIDS OF PEANUTS GROWN IN JAMAICA (Everal A. Miller and B. Singh)

The objective of this study was to determine proximate composition, amino acids and fatty acids of ten advanced peanut lines and Valencia (a variety commonly grown in Jamaica) planted for agronomic evaluation during crop years 1985 and 1986. This information will be of value to plant breeders developing new lines of peanuts and also to processors as they consider modifying their methods of processing.

Peanuts for this study were grown at the Caribbean Agricultural Research and Development Institute (CARDI) experimental plots in Lawrencefield, Jamaica. A total of eleven varieties/lines in 1985 and seven varieties in 1986 were received from the CARDI scientists in Jamaica. A portion of the whole kernels of each sample variety was ground in a Wiley Mill (Arthur H. Thomas Company, Philadelphia, PA) to pass through a 3 mm mesh screen for moisture determination. The remaining meal was stored in a refrigerator at 4.4°C for further analysis. Nutrient determinations were done using standard analytical procedures.

Proximate analyses including moisture, protein, crude fiber, and ash were conducted on the peanut kernels (meal) using standard AOCS official methods (1973). Protein was determined using the factor 5.46 ( $N \times 5.46$ ). Crude fat was determined by the AOCS official methods (1973) with the following modifications: (a) the quantity of ground sample used was 2-3 g and (b) extraction was done using the Goldfish extractor (Labconco Corporation, Kansas City, MO) with 50 ml petroleum ether for 6 hr. extraction. Total carbohydrate value was calculated using the formula  $[100 - (\text{moisture} + \text{crude protein} + \text{crude fat} + \text{crude fiber} + \text{ash})]$ .

For amino acid analysis, the peanut meal was defatted with petroleum ether and then hydrolyzed with 6 N HCL. Analysis of amino acids was carried out using the Dionex Amino Acid Analyzer Model-502 (Dionex Corporation-Houston, TX). Fatty acids were determined using essentially the procedure described in Quality Methods (APRES, 1983). Fatty acid methyl esters were prepared as described in Quality Methods (APRES, 1983). The Varian Model 3700 gas chromatograph with flame ionization detector (FID) (Varian Instrument Division, Hansen Way, Palo Alto, CA) was hooked to the Perkin-Elmer-7500 data station and a Perkin-Elmer 7500 printer plotter (Perkin-Elmer, Nowalk, CT) to obtain data. The gas chromatograph operating conditions were determined after several trials and the best resolution was obtained by the column SP-2330 with specification presented in Table 12. The peaks were identified by relative retention times of fatty acid methyl esters composite standard 21A (Nu-Check-Prep, Inc., Elysian, MN). The retention times for the composite with the chromatogram are shown in Table 13 and Fig. 2, respectively, and the chromatogram of a typical sample is presented in Fig. 3. The chromatogram from Quality Methods (APRES, 1983) was used as a reference. The percent of each fatty acid was calculated from the ratio of each peak area (printer plotter count) to the sum of areas (printer plotter count) under all peaks and reported as percent of lipid using the external standard method.

Results for this study were analyzed as a randomized complete block design with one level of nesting using each season as a complete block. Statistical analyses were performed using Statistical Analysis System (SAS, 1985). Data were analyzed using Analysis of Variance (ANOVA) procedures followed by the Duncan Multiple Range Test to compare the means.

The results of proximate composition of peanut meal for the two growing seasons (1985) and (1986) are presented in Tables 14 and 15. The first season (1985) the commonly grown variety, Valencia, had a fat content of 41.96%. The highest fat content was found in ICRISAT V13 and PF 31360 both with 43.22%, followed by ICRISAT ICG#7886 (42.71%), Altika (42.70%), Florunner (41.50%), NC#2 (41.31%), VA Bunch G2 (40.30%), NC#7 (39.54%), and Florigiant (39.51%). The protein content varied from 23.87% to 28.75% with ICRISAT V13 the lowest (23.87%) and VA Bunch G2 (28.75%) with the highest. Moisture content varied from 5.44% to 9.18%. The ash content varied from 2.93% to 2.57%; crude fiber from 1.52% to 2.93% and total carbohydrates from 17.24% to 22.62%. For the second season (1986) only seven varieties were received and analyzed. These values are presented in Table 15. The commonly grown variety, Valencia, had a fat content of 44.41%. The highest fat content was found in ICRISAT ICG#7886 (45.47%), followed by Altika (42.68%), VA Bunch G2 (42.21%), NC#7 (40.52%) and Florunner (39.24%). The protein content varied from 26.78% to 28.62% with Florunner with the lowest (26.78%), and Comet with the highest (28.62%). Moisture content varied from 5.93% to 7.29%; ash from 1.92% to 2.56% and total carbohydrates from 16.86% to 22.30%.

**Table 12. Conditions during gas chromatographic analysis of peanut oil methyl esters**

Condition	Detector Flame Ionization
Column	Coiled glass 6ftx2 10% SP2330
Liquid phase	Diethylene succinate
Solid support	80/100 chromosorb WAW
Carrier gas	Nitrogen, 60 p.s.i, 20 ml / min
Air	60 p.s.i, 300ml / min
Hydrogen	40 p.s.i, 30 ml / min
Column condition	250 Initial hold isotherm for 16 hr.
Temperature programming	Initial 224° C for 0 min, 3° C / min, final 260 C° for 0 min
Total time	12 min
Attenuation	$32 \times 10^{-10}$
Injector temperature	270° C
Detector temperature	280° C
Sample injected	.1 $\mu$ l

Table 13. Retention times of various fatty acids

Fatty * Acids	1	2
16:0	1.75	1.46
18:0	2.81	2.36
18:1	3.09	2.58
18:2	3.49	2.90
20:0	4.38	3.73
20:1	4.76	4.03
22:0	6.58	5.72
24:0	9.32	8.31

\* Number before colon indicates the chain length of the fatty acids, and number after indicates the number of double bonds.

1= Retention times for 1985 injection of standard (min)

2= Retention times for 1986 injection of standard (min)

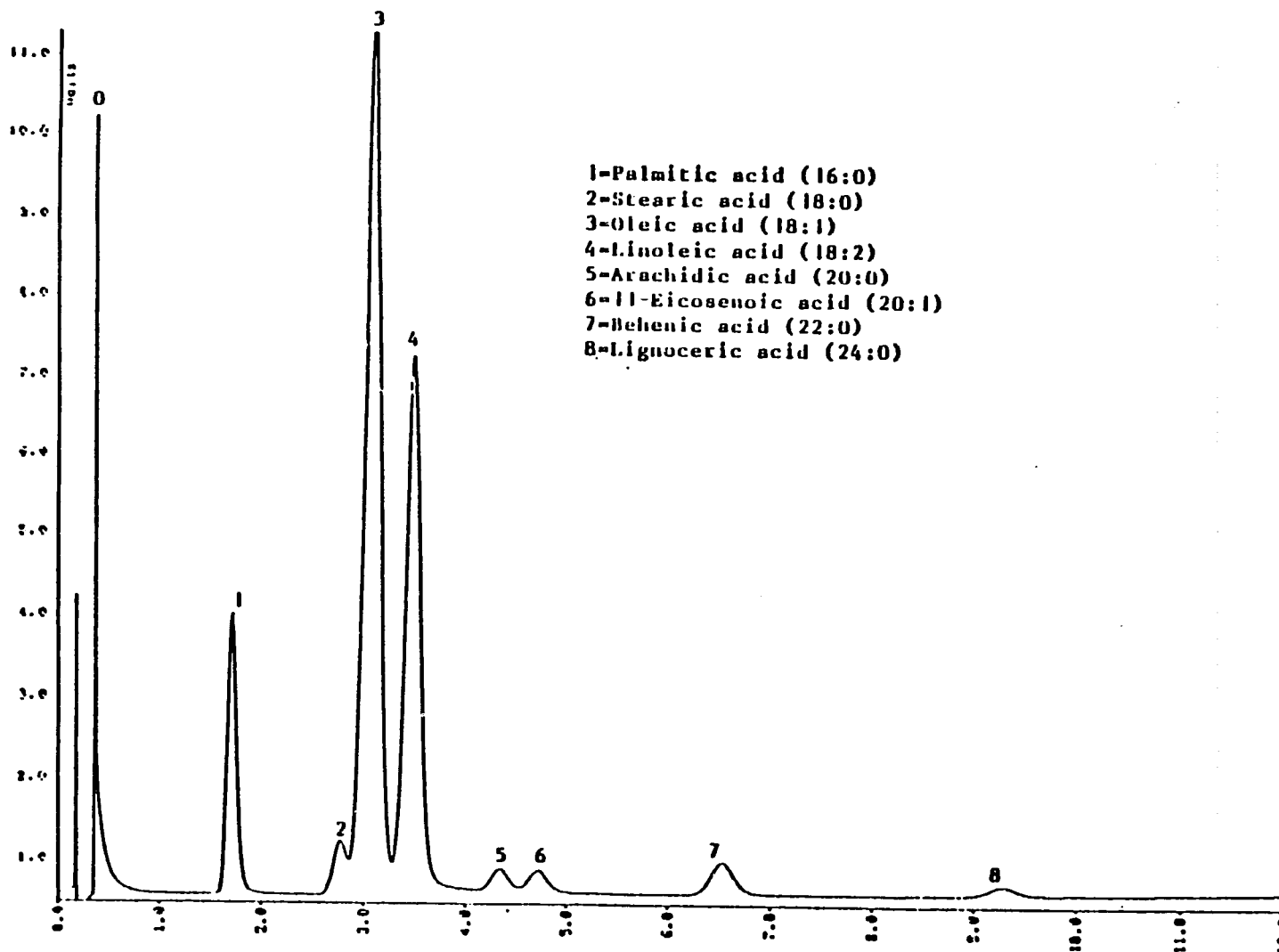


Fig. 2. Gas liquid chromatogram of standard fatty acid methyl esters.



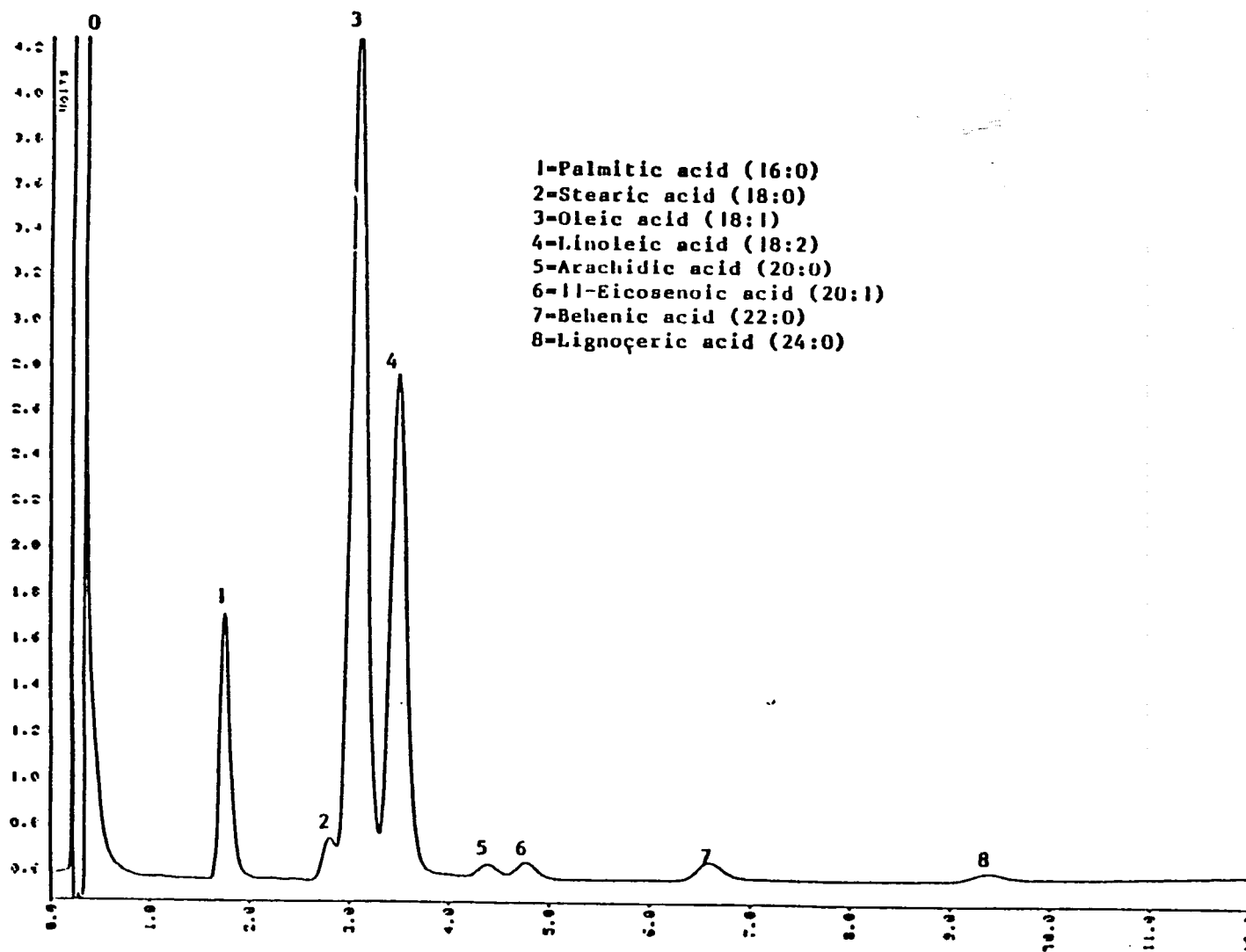


Fig. 3. A typical gas liquid chromatogram of fatty acid methyl esters in peanuts (Altika, 1985-1986).

**Table 14. Proximate composition of varieties/lines of peanuts grown in Jamaica during the 1985 Crop Year**

Varieties/ Lines	Moisture (%)*	Crude *** Protein (%)	Crude*** Fat (%)	Ash *** (%)	Crude*** Fiber (%)	CHO *** (%)
Altika	6.58cd**	28.26ab	42.70a	2.43c	1.87d	18.16f
NC#7	6.89c	27.22bcd	39.54d	2.34d	2.15c	21.86ab
VA. Bunch G2	7.37b	28.75a	40.30c	2.57a	2.07cd	18.94de
Comet	7.46b	27.95abc	39.26d	2.27e	1.89d	21.17abc
ICRISAT ICG #7886	5.64e	25.95d	42.71a	2.17f	1.56e	21.97ab
NC#2	6.12d	27.70abc	41.31b	2.31d	2.22c	20.30bcd
Florunner	6.53cd	27.08bcd	41.50b	2.26e	2.14c	20.48bcd
PF 31560	5.44e	26.56cd	43.22a	2.06g	2.93a	19.78cde
Florigiant	6.20d	26.86bcd	39.51d	2.31d	2.49b	22.62a
ICRISAT V13	6.47cd	23.87e	43.22a	2.48b	2.47b	21.49abc
Valencia	9.18a	27.85abc	41.96b	2.43e	1.52e	17.24f

\* Values given are averages of two determinations.

\*\* Means which have the same letter (abcdef) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha = 0.05 probability.

\*\*\* = As is basis

CHO\*\*\*=Carbohydrates

**Table 15. Proximate composition of varieties/lines of peanuts grown in Jamaica during the 1986 Crop Year**

Varieties/ Lines	Moisture (%)*	Crude*** Protein (%)	Crude*** Fat (%)	Ash*** (%)	Crude*** Fiber (%)	CHO*** (%)
Altika	6.22d**	27.73cd	42.68c	2.56a	1.94c	18.87c
NC #7	5.93e	28.35ab	40.52d	2.09de	2.15b	20.96b
VA Bunch G2	5.98e	28.41ab	42.21c	2.12cd	2.39a	18.88c
Comet	6.84b	28.62a	39.40e	2.27bc	2.03b	20.84b
ICRISAT ICG# 7886	6.32d	28.08bc	45.47a	1.92e	1.36d	16.86d
Florunner	7.29a	26.73e	39.24e	2.05de	2.34a	22.30a
Valencia	6.51c	27.42d	44.41b	2.35b	1.93c	17.38d

\* Values given are averages of two determinations.

\*\* Means which have the same letter (abcde) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha =0.05 probability.

\*\*\* = As is basis

CHO\*\*\* = Carbohydrates

The mean values of the two seasons are presented in Table 16. There were statistically significant differences between varieties. The highest fat content (44.09%) and a protein content of (27.01%) was observed in ICRISAT ICG#7886. The lowest percentage of oil was found in Comet (39.33%), and the protein content of that line was 28.28%. The highest protein content was found in VA Bunch G2 (28.58%). ICRISAT ICG 7886 was found to be the top line for oil (44.08%) over the two growing seasons, and VA Bunch G2 was the top line for protein (28.58%). All these varieties with the exception of VA Bunch G2 and Florunner for the two seasons demonstrate the complementary relationship rule between oil and protein: as the protein content increases oil content decreases and visa versa. The overall results perhaps would have been different if all sample varieties were available in the second growing season since ICRISAT V13 had the highest fat content and the lowest protein content in the first season. It might be predicted, based on the fat content of the closely related line ICRISAT ICG#7886, that ICRISAT V13 would also repeat its values in the second season of being the top line for fat. These two lines were developed by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) for high fat content for the tropics.

Figs. 4, 5, and 6 show graphically the moisture, protein and fat contents in peanut cultivars grown in 1985 and 1986. Statistically, there are differences in some but not all components within each variety from year to year. ICRISAT ICG #7886 showed the greatest differences for protein (Fig. 5) and Valencia and ICRISAT showed the greatest differences for oil (Fig.6). The seasonal differences for ICRISAT ICG#7886 were 2.13% and 2.76% for oil and for Valencia it was negligible for protein (0.08%) and for fat (2.45%). Valencia is a local Jamaican variety presently in use for the making of peanut butter. This variation in oil content from season to season could be one of the major problems peanut butter processors are having in Jamaica with the final product.

Amino acid composition for the varieties analyzed for the two growing seasons (1985 and 1986) are presented in Tables 17 and 18, respectively. For the 1985 growing season, it was observed that a wide variation in amino acid contents is due to differences among varieties. This trend was also observed for the 1986 growing season (Table 18) with indicated amino acid level differences among varieties. The mean value of two seasons for amino acid composition is presented in Table 19. Tables 20 and 21 present the essential but often deficient amino acids found in peanuts for the 1985 and 1986 seasons, respectively. The three limiting amino acids (lysine, methionine, and threonine) for the 1985-1986 season (Table 20) differ among varieties. The lysine content ranged from 4.45% to 7.30% with the highest found in NC#7 (7.30%) and the lowest in PF 31560 (4.45%). For methionine the range was from 0.6% to 1.80% with the highest found in Valencia (1.80%). With the exception of PF 31560 and Valencia all other varieties/lines were deficient with respect to FAO standard. For threonine the range was from 3.20 to 4.35% with the lowest in Altika and NC#7 (3.20%) and the highest (4.35%) in ICRISAT V13 and ICRISAT ICG #7886. Table 21 presents the values for the 1986 season. For lysine the range was from 6.65% to 7.60% with the highest in Valencia (7.60%) and the lowest in Altika (6.65%). For methionine the range was from 0.55 to 1.05% (all values lower than the FAO standard) and for threonine the range was from 3.05 to 3.65% (all these values exceed the FAO standard). Table 22 presents the mean values for these essential and limiting amino acids. When these values were compared with the FAO (1970) standards it was found that methionine, with the exception of the values for Valencia and PF 31560, is below the values of the FAO standards. Threonine was also higher in Jamaican peanuts as compared to the FAO values.

These results mean that peanuts grown in Jamaica may be superior in their amino acid profile with regards to the essential amino acids. Nevertheless, they are still deficient in terms of methionine. Based on the protein quality of Jamaican peanuts, as indicated by amino acid profiles, it would be interesting to see how Jamaican peanuts would perform in comparison with other peanuts in experimental animals.

**Table 16. Mean values of proximate composition of varieties/lines of peanuts grown in Jamaica during the 1985-1986 Crop Years**

Varieties/ Lines	Moisture (%)*	Crude*** protein (%)	Crude*** Fat (%)	Ash*** (%)	Crude*** Fiber (%)	CHO*** (%)
Altika	6.40bc**	27.99ab	42.67ab	2.49a	1.91f	18.51de
NC#7	6.41bc	27.78abc	40.03cd	2.21bcd	2.15de	21.41ab
VA Bunch G2	6.68abc	28.58a	41.26bc	2.35ab	2.23dc	19.91cde
Comet	7.15ab	28.28ab	39.33d	2.27abc	1.96ef	21.00abc
ICRSAT ICG #7886	5.98bc	27.01cde	44.09a	2.05d	1.46g	19.41bcd
Florunner	6.91ab	26.93cde	40.37cd	2.15bdc	2.24cd	21.39ab
Valencia	7.85a	27.64bcd	43.18a	2.30ab	1.72f	17.31e

\* Values given are averages of four determinations.

\*\* Means which have the same letter (abcdefg) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha =0.05 probability.

\*\*\* As is basis

CHO\*\*\* = Carbohydrates

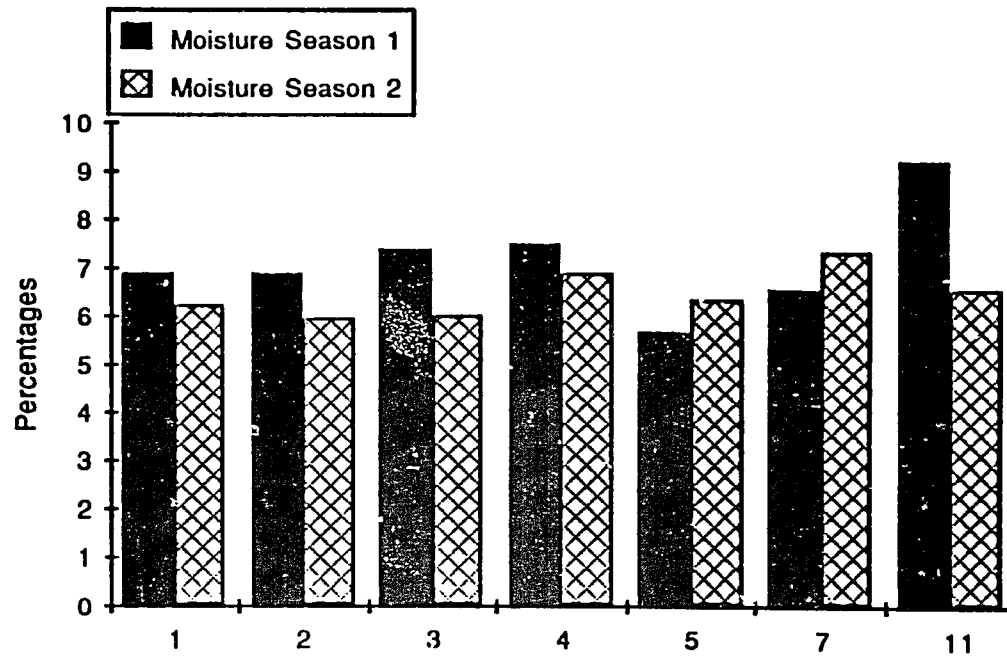


Fig. 4. Seasonal variations in moisture of varieties/lines of peanuts grown in Jamaica during 1985-1986 Crop Years.

1=Altika; 2=NC#7; 3=VA Bunch G2; 4=Comet; 5=Icrisat ICG#7886; 7=Florunner; 11=Valencia.

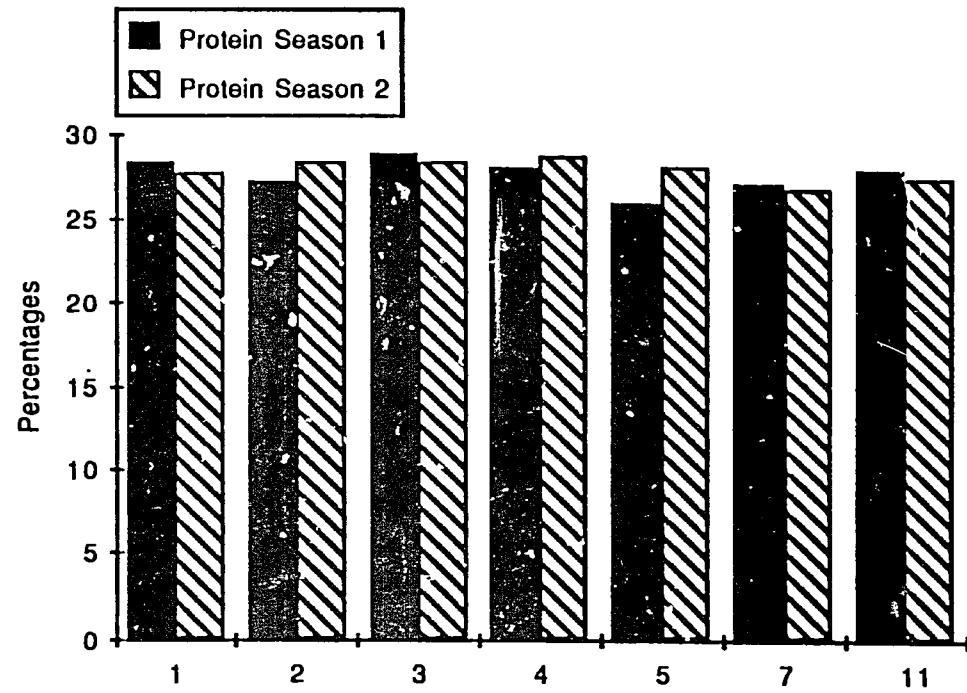


Fig.5. Seasonal variations in protein varieties/lines of peanuts grown in Jamaica during the 1985-1986 Crop Years.

1=Altika; 2=NC#7; 3=VA Bunch G2; 4=Comet; 5=ICRISAT ICG #7886; 7=Florunner; 11=Valencia.

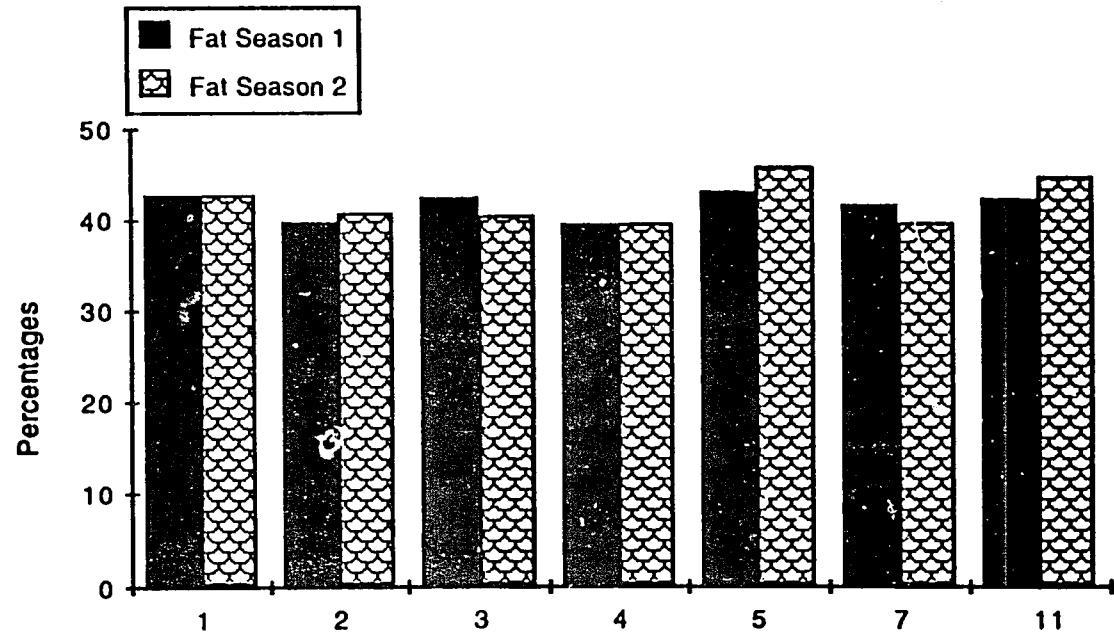


Fig. 6. Seasonal variations in fat of peanut varieties/lines grown in Jamaica during the 1985-1986 Crop Years.

1=Altika; 2=NC#7; 3=VA Bunch G2; 4=Comet; 5=ICRISAT ICG #7886; 7=Florunner  
11=Valencia.



Table 17. Amino acid composition of varieties/lines of peanuts grown in Jamaica during the 1985 Crop Year (Percent of total protein)\*

AMINO ACID	Varieties/Lines										
	1***	2	3	4	5	6	7	8	9	10	11
ASP	20.10bc**	18.80c	20.35bc	19.60bc	20.10bc	19.90bc	22.05ab	19.80bc	18.90c	23.40a	19.85bc
THR	3.20b	3.20b	3.80b	4.30a	4.35a	3.85ab	3.90ab	3.80ab	3.65ab	4.35a	4.20a
SER	8.95b	8.10b	8.00b	9.50ab	8.90b	9.40ab	9.25ab	9.35ab	9.05b	11.25a	8.35b
GLU	33.65abc	31.80b	34.60abc	33.75abc	38.25a	31.30c	37.15ab	33.50c	32.00c	37.60ab	34.05abc
PRO	6.25ab	5.70b	7.25a	6.20ab	7.20a	6.55ab	7.70a	6.20ab	6.75ab	7.70a	7.40a
GLY	9.10d	9.65dc	10.35bcd	10.20bcd	10.75abc	10.05bcd	11.70a	9.70cd	9.80cd	11.20ab	10.20bcd
ALA	6.25ab	5.85c	7.75a	6.95ab	6.10bc	5.85c	6.45bc	4.65d	6.25bc	7.75a	5.85c
CYS	0.00b	0.00b	0.25b	0.15b	0.00b	0.00b	0.00b	0.70a	0.10b	0.00b	0.00b
VAL	2.85bc	0.90c	5.20c	4.10bc	5.00b	4.45b	4.70c	3.75bc	4.00bc	4.40b	14.40a
MET	0.95c	1.00c	0.85c	0.90c	0.85c	0.60c	0.70c	1.40ab	0.65c	0.85c	1.80a
ILE	2.95b	2.95b	4.85a	4.85a	3.80ab	3.40ab	3.70ab	3.80ab	3.55ab	3.80ab	4.15ab
LEU	9.65c	9.20c	10.95abc	9.85abc	11.65a	10.00abc	11.35ab	10.50abc	9.95abc	11.00abc	11.40ab
TYR	5.55b	5.15b	6.50b	5.30b	6.25b	5.45b	6.25b	5.95b	5.25b	9.40a	5.70b
PHE	9.40a	8.85ab	7.25bc	7.85abc	7.60abc	6.50c	7.05c	6.90c	6.45c	7.95abc	7.60abc
HIS	4.15ef	3.95fg	5.30ab	3.85fg	3.75fgh	4.65cd	5.05bc	3.35h	4.55de	5.50bc	3.60gh
LYS	7.25a	7.30a	5.00bc	5.95b	4.70bc	4.65bc	5.30bc	4.45c	5.20bc	5.00bc	4.85bc
NH4	1.90d	2.00d	1.70d	1.85d	3.35ab	3.00bc	3.35ab	3.05bc	2.95c	3.60a	3.35ab
ARG	19.30bc	19.10bc	22.45a	18.00c	19.70bc	18.05bc	19.75bc	18.50bc	18.40bc	20.75ab	20.35abc

\*Values given are averages of two determinations.

\*\*Means which have the same letter (abcdefgh) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha = 0.05.

\*\*\* Numbers 1= Altika; 2 = NC#7; 3 = VA Bunch G2; 4 = Comet; 5 = ICRISAT ICG#7886; 6 = NC#2; 7 = Florunner; 8 = PF 31560  
9 = Florigiant; 10 = ICRISAT V13; 11= Valencia

Table 18. Amino acid composition of varieties/lines of peanuts grown in Jamaica during the 1986 Crop Year (Percent of total protein)\*

AMINO ACID	Varieties/Lines							
	1***	2	3	4	5	7	11	
ASP	17.30d**	16.85d	18.45cd	17.95cd	19.20bc	20.95a	20.25ab	
THR	3.05b	3.05b	3.25ab	3.30ab	3.40ab	3.55a	3.50a	
SER	7.40c	7.40c	8.20c	8.30ab	9.10a	9.00ab	9.05a	
GLU	28.65c	28.75c	30.20bc	30.40bc	32.15b	35.00a	34.75b	
PRO	6.10c	6.15c	6.35c	6.60bc	7.05b	7.70a	7.00ab	
GLY	7.65c	8.75b	8.20bc	8.55bc	8.75b	9.80a	9.20ab	
ALA	5.50cd	5.40d	5.70cd	5.75cd	5.95bc	6.60a	6.30a	
CYS	0.50a	1.45a	0.50a	0.55a	0.70a	0.90a	0.75a	
VAL	4.70a	3.65a	4.65a	4.15a	6.10a	4.80a	5.00a	
MET	0.85a	1.05a	0.65a	0.75a	0.55a	0.85a	1.05a	
ILE	3.05cd	3.20cd	3.30cd	3.00d	3.55bc	4.10a	3.80ab	
LEU	8.80d	8.85d	9.45cd	9.10cd	9.90bc	10.90a	10.50ab	
TYR	4.90d	4.80d	4.95d	6.65a	5.35c	5.95b	5.90a	
PHE	8.40ab	9.20ab	6.95c	7.05c	7.15c	9.10ab	10.30a	
HIS	3.70a	3.70a	3.65a	4.25a	3.60a	4.40a	4.20a	
LYS	6.65a	7.00a	7.45a	7.35a	7.30a	7.00a	7.60a	
NH4	0.95b	2.30b	2.45b	2.20b	3.20a	2.35b	2.25b	
ARG	17.60a	17.35a	18.00a	17.05a	15.65a	19.60a	20.20a	

\* Values given are averages of two determinations

\*\* Means with the same letter (abcd) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha = 0.05 probability.

\*\*\* Numbers 1 = Altika; 2 = NC#7; 3 = VA Bunch G2; 4 = Comet; 5 = ICRISAT ICG#7886; 7 = Florunner; 11 = Valencia.

Table 19. Mean values of amino acid composition of varieties/lines of peanuts grown in Jamaica during the 1985-1986 Crop Year. Percent of total protein\*

AMINO ACID	Varieties / Lines							
	1***	2	3	4	5	7	11	
ASP	18.70cd**	17.83d	19.40cd	18.75cd	19.65c	21.50b	20.05bc	
THR	3.13c	3.13c	3.50bc	3.80b	3.88b	3.78b	3.85b	
SER	8.18bc	7.75c	8.13bc	8.90bc	9.00bc	9.13b	8.70bc	
GLU	31.15a	30.28a	32.40a	32.08a	35.20a	36.08a	26.55a	
PRO	6.18d	5.93d	6.80abcd	6.40bcd	7.13abc	7.70a	7.20ab	
GLY	8.38e	9.20d	9.28dc	9.38dc	9.75bcd	10.75a	9.70ab	
ALA	5.88bc	5.63c	6.73b	6.35bc	6.03bc	6.53bc	6.08bc	
CYS	0.25a	0.73a	0.38a	0.35a	1.93a	0.45a	0.38a	
VAL	3.78b	2.28b	4.93b	4.13b	5.55b	4.75b	9.70a	
MET	0.90b	1.03ab	0.75b	0.83b	0.70b	0.78b	1.43a	
ILE	3.00a	3.08a	4.08a	3.93a	3.68a	3.90a	3.98a	
LEU	9.25f	9.18f	10.20cde	9.48ef	10.78bcd	11.13bc	10.95a	
TYR	5.23bc	5.00c	5.73bc	5.98abc	5.80bc	6.10ab	5.80bc	
PHE	8.90a	8.60ab	7.10abc	7.45abc	7.38abc	8.08abc	8.95a	
HIS	3.93cde	3.75de	4.48bcd	4.05bcde	3.68e	4.73b	3.90cde	
LYS	6.95ab	7.25a	6.23cd	6.65bc	6.00d	6.15cd	6.23cd	
NH4	1.43d	2.15c	2.08c	2.03c	3.28ab	2.85b	2.80b	
ARG	18.45bc	18.23bc	20.23ab	17.35c	17.70c	19.68abc	20.28ab	

\* Values given are averages of four determinations

\*\* Means with the same letter (abcdef) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha = 0.05 probability.

\*\*\* Numbers 1 = Altika; 2 = NC#7; 3 = VA Bunch G2; 4 = Comet; 5 = ICRISAT ICG #7886; 7 = Florunner; 11 = Valencia

**Table 20. Composition of the limiting essential amino acids in Jamaican peanuts (1985 Season)**

Varieties/ Lines	Percent of total protein		
	Lysine	Methionine	Threonine
Atika	7.25a**	0.95c	3.20b
NC#7	7.30a	1.00c	3.20b
VA Bunch G2	5.00bc	0.85c	3.80ab
Comet	5.95b	0.90c	4.30a
ICRISAT ICG #7886	4.70bc	0.85c	4.35a
NC#2	4.65bc	0.60c	3.85ab
Florunner	5.30bc	0.70c	3.90ab
PF 31560	4.45c	1.40ab	3.80ab
Florigiant	5.20bc	0.65c	3.65ab
ICRISAT V 13	5.00bc	0.85c	4.35a
Valencia	4.85bc	1.80a	4.20a
FAO Standard*	3.75	1.22	2.77

\* FAO (1970). Food and Agricultural Organization, Rome, Italy

\*\* Means which have the same letter (abc) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha=0.05 probability.

**Table 21. Composition of the limiting essential amino acids  
In Jamaican peanuts (1986 Season)**

Varieties Lines	Percent of total protein		
	Lysine	Methionine	Threonine
Altika	6.65a**	0.85a	3.05b
NC#7	7.00a	1.05a	3.05b
VA Bunch G2	7.45a	0.65a	3.25ab
Comet	7.35a	0.75a	3.30ab
ICRISAT ICG #7886	7.30a	0.55a	3.40ab
Florunner	7.00a	0.85a	3.65a
Valencia	7.60a	1.05a	3.50a
FAO* Standard		1.22	2.77
	3.75		

\* FAO (1970). Food and Agricultural Organization , Rome, Italy

\*\* Means with the same letter (ab) are not significantly differently different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha = 0.05 probability.

**Table 22. Mean values of the limiting essential amino acids of varieties/lines of peanuts grown in Jamaica 1985-1986 growing seasons.**

Varieties Lines	Percent of Total Protein		
	Lysine	Methionine	Threonine
Altika	6.95ab**	0.90b	3.13c
NC#7	7.25a	1.03ab	3.13c
VA Bunch G2	6.23cd	0.75b	3.50bc
Comet	6.65bc	0.83b	3.80b
ICRISAT ICG #7886	6.00d	0.70b	3.88b
Florunner	6.15cd	0.78b	3.78a
Valencia	6.23cd	1.43a	3.85b
FAO *	3.75	1.22	2.77
Pancholy et al.(1978)	2.14-3.83	0.35-0.99	3.83-4.97
Young et al. (1973)	2.88-4.45	0.71-1.21	2.01-2.73

\* FAO (1970) Food and Agricultural Organization, Rome, Italy

\*\* Means with the same letter (abcd) are not significantly different unless they bear different letters as determined by Duncan's Multiple Range Test with an alpha =0.05 probability.

Fatty acid values and the oleic / linoleic (O/L) ratio for the two growing seasons 1985-1986 and 1986 are presented in Tables 23 and 24, respectively. The 1985 growing season showed significant differences among varieties in some but not all fatty acids. Oleic acid and linoleic acid ranges were significant. For oleic acid the range was from 38.35% to 51.39% with the highest found in NC#7 (51.39%) and the lowest in Comet (38.35%). The linoleic acid content ranged from 29.90% to 39.57% with the highest found in Valencia (39.57%) and the lowest in NC#7 (29.90%). The O/L ratio showed a range from 0.98% to 1.72% with the highest in NC#2 (1.72%) and the lowest in ICRISAT ICG#7886 (0.98%). For the 1986 growing season the results of the seven varieties analyzed are presented in Table 24. The oleic acid ranged from 41.27% to 56.25% with the highest found in VA Bunch G2 (52.25%) and the lowest in ICRISAT ICG#7886 (41.27%). For linoleic acid the range was from 27.90% to 38.16% with the highest found in Valencia (38.16%) and the lowest found in Altika (27.90%). The O/L ratio ranged from 1.04% to 1.95%, with the highest found in NC#7 and the lowest in Comet 1.04%. Mean values for fatty acid for both growing seasons and varieties are presented in Table 25 along with their respective O/L ratio.

The varieties in Jamaica showed variation for oleic acid from 41.99% to 52.16% and for linoleic acid 28.84% to 39.53% for the mean values (1985-1986). The O/L ratio of Jamaican peanuts ranged from 1.02% to 1.77% with Altika with the highest ratio (1.77%) and Comet the lowest (1.02%). Levels of polyunsaturated fatty acids are inversely proportional to the keeping quality of an oil. Therefore, the lower the O/L ratio the better is the keeping quality of the oil. These results would give the peanut breeders in Jamaica an indication of the genotype to develop that would be satisfactory to the manufacturer who is especially interested in the stability of the final product. It also becomes a task of the peanut breeder to develop a line with a high P/S ratio for the nutritionally conscious public.

Table 26 presents the saturated fatty acids (palmitic, C16:0, stearic C18:0, arachidic C20:0, behenic C22:0, and lignoceric C24:0); the unsaturated fatty acids (oleic C18:1, linoleic C18:2, and eicosenoic C20:1), along with the calculated polyunsaturated to saturated ratio (P/S) ratio

#### PLAN OF RESEARCH FOR 1988-1989

- (1) Completion of the work on maturity aspects of the peanuts grown in the eastern Caribbean region.
- (2) Completion of the Ph.D. thesis of Ms. Margaret Hinds.

Table 23. Fatty acids of oil from varieties/lines of peanuts grown in Jamaica during the 1985 Crop Year

Fatty Acid Composition (% of lipid)**											
FATTY ACID	1***	2	3	4	5	6	7	8	9	10	11
PALMITIC	11.13de**	11.19de	10.75e	14.35a	14.38a	10.59e	11.76de	12.26be	11.75de	10.43e	12.94b
STEARIC	2.19b	2.00b	1.53dc	2.66a	1.41d	1.87bc	1.58dc	1.57dc	1.26d	1.45d	2.14b
OLEIC	49.93a	51.39a	50.03a	38.35f	38.73f	47.57b	50.08dc	43.02de	43.98dc	45.98bc	41.50e
LINOLEIC	31.08cd	29.90d	31.73cd	38.18a	39.65bc	34.00bc	34.17bc	38.35a	36.25cd	39.83a	39.57a
ARACHIDIC	1.50ab	1.34ab	1.29ab	1.56a	0.98cd	1.32ab	1.30ab	0.80d	1.30ab	1.23bc	1.23bc
EICOSENOIC	1.59ab	1.35bcd	1.54ab	1.23de	1.45abcd	1.49abc	1.63a	1.16e	1.55ab	1.50abc	1.26cde
BEHENIC	1.78e	2.20bcd	2.05de	2.54abc	2.48abcd	2.13cde	2.69a	1.90e	2.63ab	2.40abcd	2.39abcd
LIGNOCERIC	0.81bc	0.64cd	1.09ad	1.11ab	0.92bc	0.98abc	1.10ab	0.93abc	1.28a	0.81bc	0.41b
O/L Ratio	1.61	1.72	1.57	1.00	0.98	1.40	1.47	1.12	1.21	1.16	1.05

\*\* Values given are averages of three determinations.

\* Means which have the same letter (abcdefg) are not significantly different unless they bear the same letters as determined by Duncan's Multiple Range Test with an alpha = 0.05 probability.

O:L=Oleic: Linoleic ratio

\*\*\* Numbers 1=Altika; 2=NC#7; 3=VA bunch G2; 4=Comet; 5=ICRISAT ICG#7883; 6=NC#2; 7=Florunner; 8=PF 31560; 9=Florigiant; 10=ICRISAT V13; 11=Valencia



Table 24. Fatty acids of oil from varieties/lines of peanuts grown in Jamaica during the 1986 Crop Year.

Fatty Acid Composition (% of lipid)**							
FATTY ACID	1	2	3	4	5	7	11
PALMITIC	10.82de	10.98d*	10.44e	14.65a	15.00a	13.96b	13.29c
STEARIC	1.96ab	2.01ab	1.87ab	2.02a	2.10a	1.40ab	1.83ab
OLEIC	54.39b	52.84c	56.25a	39.53f	41.27e	43.42b	42.48de
LINOLEIC	27.90e	29.84d	25.95f	37.89a	36.66h	35.61c	38.16a
ARACHIDIC	1.21a	1.90a	1.22a	1.43a	1.07a	1.06a	1.34a
EICOSENOIC	1.61a	1.28b	1.22b	1.25b	1.55a	1.45a	1.28b
BEHENIC	2.45c	2.48c	2.06d	3.18a	2.36bc	2.80b	2.68bc
LIGNOCERIC	0.98abc	0.85bc	0.96abc	1.01ab	1.05ab	1.18a	0.76c
O/L Ratio	1.95	1.77	2.17	1.04	1.13	1.22	1.11

\*\* Values given are averages of three determinations.

\* Means which have the same letter (abcdefg) are not significantly different unless they bear the same letters as determined by Duncan's Multiple Range Test with an alpha = 0.05 probability.

O:L=Oleic: Linoleic ratio

\*\*\* Numbers 1=Altika; 2=NC#7; 3=VA bunch G2; 4=Comet; 5=ICRISAT ICG#7886; 7=Florunner; 8=PF 31560; 11= Valencia.

**Table 25. Mean values of fatty acids of oil from varieties/lines of peanuts grown in Jamaica during the 1985-1986 Crop Years.**

Fatty Acid Composition (% of lipid)**							
FATTY ACID	1***	2	3	4	5	7	11
PALMITIC	10.97de*	11.08d	10.60de	14.50a	14.69a	12.86b	13.12b
STEARIC	2.08ab	2.00abc	1.70bcde	2.40a	1.37e	1.49de	1.99abc
OLEIC	52.16a	52.12a	53.14a	38.94g	40.00g	46.75bc	41.99ef
LINOLEIC	29.49e	29.87e	28.84e	38.04ab	38.16ab	34.89cd	38.87a
ARACHIDIC	1.35a	1.26ab	1.25ab	1.35a	1.02bc	1.18ab	1.28ab
EICOSENOIC	1.60a	1.27cde	1.38bcd	1.24de	1.50abc	1.54ab	1.27de
BEHENIC	2.12def	1.32cde	2.06f	2.86a	2.52bc	2.75ab	2.54bc
LIGNOCERIC	0.89bcd	2.34de	1.03abc	1.06abc	0.99bcd	1.14ab	0.58e
O/L Ratio	1.77	0.75	1.84	1.02	1.05	1.35	1.08

\*\* Values given are averages of six determinations

\* Means which have the same letter (abcdefg) are not significantly different unless they bear the same letters as determined by Duncan's Multiple Range Test with an alpha = 0.05 probability

O:L=Oleic: Linoleic ratio

\*\*\* Numbers 1=Altika; 2= NC#7; 3=VA bunch G2; 4=Comet; 5=ICRISAT ICG#7886; 7=Florunner; 11= Valencia.

**Table 26. Quality traits of varieties/lines of peanuts grown in Jamaica 1985-1986.**

Varieties/ Lines	Total saturated Fatty Acids (16:0 18:0 20:0 22:0 24:0)***	Total unsaturated Fatty Acids (18:1 18:2 20:1)	Total polyunsaturated Fatty Acids (18:2)	P/S Ratio**
Altika	17.41	83.25	29.49	1.69
NC#7	17.44	83.31	29.87	1.71
VA Bunch G2	16.64	83.36	28.84	1.73
Comet	22.17	78.22	38.04	1.72
ICRISAT ICG #7886	20.59	79.66	38.16	1.85
NC#2*	16.87	83.06	34.00	2.01
Florunner	19.4	83.18	34.89	1.80
PF 31560*	17.46	82.53	38.35	2.20
Florigiant*	18.22	81.78	36.25	2.22
ICRISAT V13*	16.32	87.01	39.53	2.42
Valencia	19.51	82.13	38.87	1.99

\* One season total

\*\* P/S = Polyunsaturated to saturated ratio

\*\*\* 16:0 = Palmitic ; 18:0 = Stearic; 20:0 = Arachidic; 22:0 = Behenic; 24:0 = Lignoceric; 18:1 = Oleic;  
18:2 = Linoleic; 20:1 = Eicosenic.

GA/FT/TP

**APPROPRIATE TECHNOLOGY  
FOR STORAGE/UTILIZATION OF PEANUT**

UNIVERSITY OF GEORGIA - THAILAND AND PHILIPPINES

LARRY R. BEUCHAT, PRINCIPAL INVESTIGATOR, UGA

**INTRODUCTION**

The initial years of this project were largely directed toward evaluating procedures for handling, sorting, packaging and storing peanuts. Objectives were to develop and demonstrate procedures to eliminate aflatoxin-contaminated kernels from lots received from farmers and to prevent growth of aflatoxigenic aspergilli on peanuts through control of temperature and equilibrium relative humidity during storage. Maintenance of sensory quality of raw and roasted kernels was also a high priority.

The original objectives of the project were achieved. In the three-year extension, we initiated a more concentrated effort toward developing and adapting technologies to utilize peanuts and peanut products in traditional and new food products. The choice of such products is based in large part on consumer survey response but also on intuition and insight to consumer behavior in Thailand, the Philippines and the U.S. The first year of the three-year extension has seen substantial progress in meeting the objectives set forth in the project.

**MAJOR ACCOMPLISHMENTS****Research****A. University of Georgia (UGA)**

The effects of acidification and glucose supplementation on fermentation of ammoniated peanut meal by *Lactobacillus bulgaricus* were studied. Peanut meal was supplemented with either 2% or 4% glucose. Each meal was then either acidified to approximately pH 6 before inoculation with *L. bulgaricus* or inoculated with the organism without acidification. Bacterial populations and changes in pH and titratable acidity were measured over a 72-h fermentation period. There appeared to be no significant differences in acid production between the treatments.

The effect of maturity and harvest time on mechanical properties of peanut kernels was studied. The hypothesis that peanut shell color can serve as a single index to group peanuts with similar mechanical properties was also tested. The hardness and brittleness of peanuts increased with harvest time and maturity. The shear and compression energy measuring using a modified Kramer cell increased with maturity at each harvest time but showed no significant differences among harvest times. Results indicate that the mechanical properties of peanuts can be predicted from the maturity distribution of those peanuts.

Our efforts to develop a paste containing > 90% unroasted peanuts continues. The ultimate use of this paste flavored with cheese, meat, fruit or chocolate would be in the form of a spread or filling for bread, crackers and confectionery items. The quality of pastes prepared from peanut kernels subjected to hot water treatment, steam and dry heat were compared. Results indicate that hot water treatment produces a paste with lightest color and most bland flavor.

The effect of particle size reduction on quality of pastes prepared from whole kernels subjected to hot water treatment was determined. Results indicate that paste prepared from peanuts chopped to a particle size of 3.36 to 4.75 mm exhibited to most desirable sensory qualities.

The flavor of pastes prepared from whole and chopped peanuts which had been subjected to heat treatment was compared with two commercial peanut butters and a paste made from raw untreated peanuts (control). Cluster analysis of results revealed that the premium brand of peanut butter had the strongest roasted flavor while the pastes had significantly more beany flavor than the peanut butters but less than that in the raw control. Chopping kernels results in the loss of roasted and sweet flavors and the introduction of cardboard, musty and metallic flavors.

Textural characteristics of two peanut butters and one peanut paste were determined using three instrumental methods and a texture profile panel. Instrumental measures show that peanut paste had the hardest texture, required the greatest adhesive force but the least time to break a peanut column, and was least cohesive. All instrumental methods were acceptable for evaluating the textural properties of the products but texture profile analysis provided more information to describe texture. The panel rated the paste as firmest, least adhesive, spread least on the tongue, least smooth, most grainy, and most difficult to swallow. Significant correlations were found between instrumental measures and sensory characteristics of spread, hardness and adhesiveness.

#### B. Kasetsart University (KU)

Research to develop a highly acceptable formulation for a peanut butter bar continues at KU. An improved formulation consists of 72% fine ground roasted peanuts, 12% maltose syrup, 9.5% fine ground sugar, 3% roasted desiccated coconut, 2% fine roasted rice, 1% roasted sesame seed and 0.5% salt. Sensory evaluation by consumers revealed that 57% rated the product at a high level; 73.7% indicated they would buy the product.

A spread containing a mixture of 20% fruit (banana, papaya and durian) powders and 80% peanut butter was highly acceptable. Sensory analysis showed that panelists preferred the banana-peanut butter spread. Additional formula modification and shelf life tests are warranted.

A chocolate flavored peanut beverage containing 3.5% peanut protein isolate, 3.5% fat, 8% sugar, 0.7% cocoa powder, 0.1% stabilizer and water was highly acceptable to fifty-five untrained panel members. The majority of panelists were willing to pay 4.5 bahts for 250 ml of beverage. Larger scale consumer tests are needed to confirm the acceptance of the product. Formulation of a milk-shake type product based on peanut protein isolate may also be acceptable to Thais.

Sensory qualities of ground chicken extended with defatted peanut flours were determined. Cooking losses and shrinkage of extended ground chicken patties were less than those of control patties. Peanut flour increased water retention capacity. Pure chicken patties were less tender than extended patties. Problems associated with color, flavor and odor of extended patties exist; however, there was no difference in overall preference scores among the control and extended patties containing 15 and 20% DPF and 10% PDPF.

#### C. University of the Philippines at Los Banos (UPLB)

A total of 84 samples of peanut products (representing 18 different products) were collected from the 13 regions of the Philippines. The source, region, province and manufacturer's name and address were recorded. A survey was also conducted to determine different uses of peanuts as food ingredients. Sensory evaluation of the 15 different products was done. Evaluation of each product included the product description and results of sensory evaluation. Improvement of several products will be based on information from the survey.

A total of 142 peanut samples (58 raw peanut seeds and 18 different peanut products consisting of 84 samples), collected from various regions in the Philippines were analyzed for aflatoxin content. Results of the analyses showed that 55.1% of the raw peanut seed samples and 27.3% of the products were positive for aflatoxin.

Mold isolates (159) from soil, water and air were screened for their ability to inhibit *A. parasiticus*. A few strains of the *A. niger* group were capable of outgrowing *A. parasiticus* but the

latter was able to recover in three days. *Aspergillus fumigatus* was another species capable of restricting the growth of *A. parasiticus* but it can be a more potent toxin-producer. A mold isolate possessing characteristics typical of *Cladosporium fulvum* completely suppressed the growth of *A. parasiticus*. Further studies are underway to define the mechanism of inhibition and assess the efficacy and safety of using this isolate to inhibit growth of *A. parasiticus* in peanuts and peanut products.

Peanut milk prepared using 1:8 (peanut:water) ratio was used in the production of yuba (peanut film). Glycerol at 1.0 and 2.5% levels was added and its effect on strength properties of films was determined. Full-fat (1:6, 1:7, 1:8 and 1:9 [peanut:water] ratios) and partially defatted (1:8 and 1:10) milk supplemented with 2.5% glycerol were also prepared and the effect of the dilution on chemical and physical properties of films was determined. Increasing levels of glycerol caused an increase in breaking strength and a decrease in hardness of yuba. The amount of water used did not affect the chemical composition of films but tests for tensile strength revealed that the formulation with the least amount of water from both full-fat and partially defatted peanut milk produced the strongest films. Partially defatted films, although containing a higher amount of water-soluble constituents, were generally stronger than full-fat films. Scanning electron microscopy also revealed that partially defatted films have a more uniform and compact structure than full-fat films where fat globules are liberally distributed.

#### EXPECTED IMPACT OF PROJECT

Progress toward understanding the potential for success of peanut-based products under various stages of development for the Thai, Filipino and U.S. markets has been made in the past year. The Thai and Filipino populations are very receptive to a wide variety of peanut-based products.

We continue to use a several-pronged approach in all cooperating laboratories which has as its objective increased utilization of peanuts, in whatever form. Such increases would be expected to result in increased nutritive value and safety of products to the consumer.

The work on biological control of aflatoxin production by aspergilli and on detoxification of aflatoxin-contaminated peanuts is in its initial stages. However, the potential impact of successfully developing a biological method for reducing or eliminating aflatoxin from peanuts (and other agricultural commodities) will be tremendous on a global scale.

Successful fermentation of ammoniated peanut meal would allow aflatoxin-contaminated peanuts to be detoxified, fermented, and perhaps be used as animal feed. Providing such a use for the contaminated product would reduce the financial loss to farmers and processors by allowing them to use peanuts which would normally be used as fertilizer or considered useless. Moreover, the farmers and processors would have more motive to remove contaminated kernels from batches of peanuts destined for human use, thereby decreasing the risk that aflatoxins would be consumed.

#### GOAL

The goal of the project remains to enhance the capabilities of scientists, technicians and students at KU, UPLB and UGA. The training of cooperators from Thailand, the Philippines and the U. S. in developing and transferring appropriate peanut storage and utilization technologies is the mechanism by which all institutions will enhance their capabilities to improve and assist in economic and human development.

#### OBJECTIVES (1987-88)

##### A. University of Georgia

1. Optimize process for the preparation of aqueous extract (milk) from peanut kernels to be developed into non-fermented and fermented products.

2. Optimize the process for the preparation of a paste from peanut kernels, and develop products prepared from peanut paste.
3. Develop methodologies for increased efficiency in the development of value-added products from peanuts.
4. Investigate ammoniation followed by bacterial fermentation to detoxify aflatoxin-contaminated kernels, and evaluate end products as a poultry feed.

**B. Kasetsart University**

1. Survey Thai consumers for their attitudes and perceptions toward selected peanuts and peanut products.
2. Develop peanut-supplemented confectionery product acceptable to Thai consumer.
3. Determine functional properties of peanut flour as related to its behavior in chicken products.

**C. University of the Philippines at Los Banos**

1. Survey of existing peanut products in the Philippines.
2. Quality assessment of existing peanut products in the Philippines.
3. Improve/develop products from peanuts using traditional technologies.
4. Isolation, identification and screening of microorganisms for antagonistic properties against aflatoxin-producing fungi.

**ORGANIZATION**

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 Ms. Irene E. Matalog, Research Assistant

**Department of Agricultural Economics**

Dr. Romy Huelgus, Co-Investigator

**ACCOMPLISHMENTS IN DETAIL****A. Accomplishments at the University of Georgia****1. Mechanical Properties of Peanuts as Affected by Maturity and Harvest Time**

Hull scrape methods is one of the most commonly used methods for determining the harvest time of peanuts. This method was developed by investigation of the correlation between peanut hull color and maturity. We investigated mechanical property differences among peanuts from different maturity groups as determined by the hull scrape method. The effect of harvest time on mechanical properties of peanuts was also studied. Objectives were to evaluate the effect of maturity and harvest time on mechanical properties of peanuts and test the hypothesis that peanut shell color can be the single index to group peanuts with similar mechanical properties.

Peanuts (Florunner C.V.) were harvested from the Southwest Branch Station farm of the University of Georgia 110, 125, 140 and 155 days after planting. They were separated into Yellow 1 (yellow), Yellow 2 (dark yellow), Orange, Brown, and Black maturity groups by scratching the shell surface and then matching the colors on the Peanut Profile Board. Moisture content of peanuts was determined by the vacuum oven method. Mechanical parameters were derived from force—deformation curves. Kernel hardness was interpreted as the maximum force obtained from both tests. Energy used to shear and compress peanuts was calculated by integrating the area under the curve up to the maximum force with the modified Kramer cell. The slope was calculated from the linear portion of initial increase in the modified Kramer cell response and was used as an indicator of brittleness. Mechanical properties of the mixed group were evaluated by the modified Kramer cell method.

Moisture content decreased with increasing time. The maximum force required to fracture peanuts increased as moisture content decreased. In the modified Kramer cell test, the energy to compress and shear peanut increased with harvest time, but only values at 110 and 155 days were significantly different. The slope value which is considered as an index of brittleness, was significantly



higher at 155 days than at 110 and 125 days. Generally, peanuts became more brittle as harvest time increased.

Based on objective texture data, peanuts became dryer, harder, and more brittle as maturity increased. Therefore, once the standard values of force, energy and slope required to fracture, shear and compress peanuts are known, mechanical properties measured by Instron may be an indicator of peanut maturity. Peanuts with the same maturity but from different harvest times showed different physical properties. There are many environmental factors that influence peanut quality, e.g., climate, amount of rain, type of soil and fertilizer. Therefore, sorting peanuts based on their maturity groups may not guarantee uniformity. However, the energy values required to shear and compress peanuts at different harvest times were not significantly different. These values can be considered as the index of sensory crunchiness which may be a more important property to the consumer than others. More study is needed to evaluate the effect of harvest time on the textural quality of peanuts.

Peanuts are dried to approximately 8% moisture for safe storage after harvest. Mechanical properties of dried peanuts may not follow the same pattern as fresh peanuts. Further study of the chemical composition of peanuts harvested at different times with different maturities will be needed to better evaluate the effect of harvest time and maturity on the mechanical properties of peanuts. The measured mechanical properties of mixed peanuts were similar to the calculated results. This demonstrated that the mechanical properties of mixed peanuts can be predicted from the maturity distribution of those peanuts.

## 2. Fermentation of Ammonia-Detoxified Peanuts

Preliminary studies were conducted to determine optimal conditions for the use of lactic acid bacteria to ferment ammoniated peanuts or peanut meal. These results will be used in subsequent studies in which we will ferment ammoniated, aflatoxin-contaminated peanuts. Acidification of ammoniated peanut meal or addition of glucose before inoculation with *Lactobacillus bulgaricus* had no significant effect on either the titratable acidity or the pH during the fermentation. Likewise, acidification or added glucose did not affect the growth of microorganisms in meal. After fermentation, meals were dried to observe any obvious physical differences the various treatments might have caused. No apparent differences were observed between treatments. All meals were light tan in color, and had a rough texture and a slightly sweet smell. Based on these results, future studies on the fermentation of aflatoxin-contaminated peanuts will be done without acidification or glucose supplementation.

## 3. Evaluation of Texture and Color of Peanut Paste

Our efforts to develop a paste containing > 90% unroasted peanut kernels continue. The ultimate use of this paste in variously flavored, textured and colored formulations would be in the form of a spread or filling for bread, crackers and confectionery items. The objectives of one of the studies conducted in the past year was to compare chemical, instrumental and sensory measures of quality, texture and color of peanut pastes prepared by the application of three methods of processing kernels prior to preparation into the pastes. The three methods compared were the application of dry heat, steam and hot water. Lipxygenase activity was measured and observed to be eliminated by each of the three process treatments.

Dry heat treatment was administered in a convection oven by heating at 53°C for 10 min. Steam extraction was accomplished by heating at 70°C for 45 min in a steam retort. The water extraction process involved combining the peanuts with water (66°C) at a ratio of one part peanuts to six parts water. Peanuts were heated and stirred continuously for 5 min before water was decanted. Water extraction was done for 5, 20 and 20 min. Steamed and water extracted kernels were dried in a convection oven at 100°C for 1 hr, then at 37°C for 24 hr.

Moisture content of the peanuts differed significantly. The final moisture content of the paste from the dry heated peanuts was the lowest (2.5%) followed by the water extracted peanuts (3.3%). Moisture content in the paste from water extracted peanuts was reduced by 39% compared to that

from raw peanuts. The Hunter color lightness (L) value of the water extracted kernels was significantly higher than that of the kernels treated with dry heat or steam. Two instrumental measures for texture were work, expressed as the "work necessary to remove food sample from the plunger surface," and time in sec "required to break the column of paste that forms between the plunger and the sample surface." Work was not significantly different between the three treatments.

Color scores revealed that water extracted peanuts were lightest colored, and dry heated peanuts were darkest in color. Instrumental color measures, L, and sensory data for color were significantly correlated ( $r = -0.74$ ,  $P > 0.01$ ). Flavor intensity scores were lower for the pastes from water extracted nuts compared to steam treated nuts. The raw flavor intensity was not different between pastes from steam treated or water extracted kernels. The water extraction method was clearly superior in reducing the flavor intensity of the peanuts. This method also produced a lighter colored paste as indicated by low lightness scores for both instrumental and sensory measures.

#### 4. Effect of Particle Size Reduction on the Quality of Peanut Pastes

The objective of this study was to determine the effect of particle size on quality of paste prepared from peanuts which had been hot water extracted. Blanched peanuts were steamed at 100°C for 2 min to inactivate lipoxxygenase. The kernels were then chopped to particle sizes ranging from 3.36 - 4.75 mm (coarsely chopped, CC), 2.00 - 3.36 mm (moderately chopped, MC) and 1.18 - 2.00 mm (finely chopped, FC) then prepared into a paste. Paste from whole peanuts (W) served as a control.

Paste prepared from finely chopped peanuts was scored lowest in flavor intensity. It also had the lowest lightness (L) values and the lowest protein content. Flavor intensity of the paste from the CC peanuts was not significantly different from that from the FC peanuts. Paste from CC peanuts had the highest instrumental lightness (L) values and did not contain significantly less protein or oil than paste from whole kernels. Results indicated that peanuts chopped to a particle size of between 3.36 and 4.75 mm would produce a paste with most desired characteristics.

#### 5. Comparison of Flavor Quality of Peanut Pastes and Peanut Butter

This study was conducted to compare the flavor quality of peanut pastes prepared from peanut kernels subjected to a water extraction process to remove flavor precursors, with two commercial peanut butters and a paste prepared from raw peanut kernels. A panel of 12 judges was trained to evaluate the pastes. Cluster analysis and graphical representation of the cluster scores were used to compare sensory scores of the two pastes and two peanut butters. The premium brand peanut butter had the strongest roasted flavor and no beany flavor. The pastes had the lowest roasted flavor and significantly more beany flavor than the peanut butters but less than that in the raw control. There was no difference in the beany flavor of the paste made from whole or chopped peanuts, but cardboard and earthy flavors were significantly lower in pastes made from whole kernels compared to pastes made from chopped kernels. Chopping of the peanut kernels to a particle size of 2.00 to 3.36 mm did not enhance the removal of beany flavors in the paste, but resulted in loss of flavor quality through introduction of cardboard and earthy flavors.

#### 6. Characterization of Textural Properties of Peanut Paste

Peanut paste is envisioned to serve as the base ingredient for products such as imitation cheese, chocolate, meat and fruit flavored spreads. In such applications, the textural properties of the paste is of great importance. Studies were conducted to determine textural characteristics of two peanut butters and one peanut paste using three instrumental methods, textural characteristics of the three spreads using a texture profile panel and to establish a correlation between instrumental and sensory measurements.

Three methods were selected to characterize the texture of the spreads. A cone-shaped probe attached to a penetrometer was used in method A and depth of penetration was recorded. Method B involved extrapolating texture parameters from the force distance curves. Method C consisted of a modified texture profile analysis using an Instron Universal Testing Machine. A 2.5 g sample was

compressed twice by two flat plates which were covered with contact paper to a 2 mm clearance between the two plates. Texture parameters were extracted from the force-distance curves.

The three products differed in texture at the 5% level of significance. Six texture parameters were derived from method C, four from method B and only one from method A. The depth of penetration in method A, the maximum force penetration in method B and hardness in method C are all indices of hardness. Results indicate that peanut paste was hardest while the commercial brand of peanut butter was the least hard. The time required to break the peanut butter column for each of the products in method B was similar to the time recorded in method C. The least amount of time was required to break the peanut paste column. More work was needed to remove the sample from the plunger surface used in method C than in method B. The least amount of work to remove peanut paste from the plunger surface.

### 7. Quality Characteristics and Consumer Acceptance of a Peanut-Based Imitation Cheese Spread

Results of consumer tests on six formulations of peanut-based imitation cheese spreads using a consumer panel of 200 Filipinos are presented in Table 11. Mean scores for overall preference and purchase intention were not significantly different. Overall preference scores were significantly different between flavor levels and among the six formulations. Mean scores were highest, though not significantly different for the 4 and 6% level of flavoring. When individual formulations were compared, formulations containing 0.5% salt and 6% flavoring and 1% salt and 4% flavoring received the highest overall preference scores. Mean ratings of 4.0 to 4.4 correspond to the "like slightly" and "neither like nor dislike" points on the 9-point preference scale.

The peanut-based spreads contain 23.5 to 24.4% protein, nearly twice that in the dairy-based spreads. Moisture content in the peanut-based spreads was 4.2 to 4.6%, and had an  $a_w$  of 0.42. The high protein and calorie content in this product is an important justification for its further development for consumption in the Philippines. The low moisture content and microbiological stability at ambient temperature are important quality characteristics in any developing country where refrigeration of foods in the home is often not practiced.

#### B. Accomplishments at Kasetsart University

Research done in 1987-88 included a consumer test on a peanut bar and the development of three new peanut products—peanut spread with fruit powder, chocolate flavored peanut beverage and ground chicken patties extended with peanut flour. These products were tested for their qualities and consumer acceptance.

##### 1. Consumer Test on Peanut Bar

The peanut bar is a modified product consisting of a peanut butter base. Attempts were made in the past year to improve the quality of the peanut bar. The modified formulation consisted of 60% fine ground roasted peanut, 20% course ground roasted peanut, 5% fine ground roasted rice, 3% roasted desiccated coconut, 10% palm sugar and 0.5% salt. The product was oily in appearance and had a soft texture. After storage, it cracked and rapidly became rancid.

The recent improved formulation contains 72% fine ground roasted peanuts, 12% maltose syrup, 9.5% fine ground sugar, 3% roasted desiccated coconut, 2% fine ground roasted rice, 1% roasted sesame seed and 0.5% salt. All ingredients were mixed at 60°C and passed through a peanut butter mill. The mixture was then pressed into a rectangular shaped mold (25 x 30 x 10 mm). The finished product (15 g) was subjected to quality testing. The product had  $a_w$  of 0.47, a peroxide value of 0.82 to 1.20 and a total plate count of 5000 microorganism per gram. Yeast and mold population was less than 100 colonies per gram.

Consumer response to the peanut butter bar was studied. A questionnaire consisting of 100 questions was prepared emphasizing the background of the consumers, preference for the product, the

price willing to pay for the product, the packaging expected and the frequency of eating. Consumer testing was conducted at KU during the National Agriculture Fair. Two hundred and fifty-one questionnaires were randomly distributed to consumers of different backgrounds. The results showed that more than 57% of the consumers preferred this product at high to highest levels. There was not much response on the categories of like or dislike the product (19.9%). About 73.7% of the consumers showed their interest in buying the product. Some improvements in quality are needed before subjecting the product to further market testing.

## 2. Peanut Butter Spread Containing Fruit Powder

Peanut spread consists of peanut butter and other products, such as fruit jam, fruit butter, fruit paste and chocolate. The components are mixed together into a homogenous product which has a better flavor, smooth texture, and good spreadability. Products containing 25% banana and papaya and 75% peanut butter were preferred to pineapple. Replacing fruit paste with drum-dried fruit powder was investigated in the past year. This study had as its goal the development of a peanut spread containing banana, papaya and durian powders mixed with peanut butter. Durian powder was introduced in the study because it had a popular natural fruit flavor among many Thais.

The results showed that peanut spreads mixed at ratios of 1:1 (powder:peanut butter) and 1:2 were unacceptable because the products were dry and cracked. Fruit flavors were very strong and not compatible with peanut butter flavor. A ratio of 1:3 was moderately accepted, but the mixture of 20% fruit powder and 80% peanut butter (ratio 1:4) was highly acceptable. This formula had an  $a_w$  of 0.44 to 0.46, peroxide value of 1.02 to 2.32, total microbial population of less than  $10^3$  colonies per gram and yeast and mold populations of  $10^2$  colonies per gram. Sensory analysis by the QDA technique indicated that panelists preferred the banana peanut spread. They commented that the flavor of the durian/peanut spread was too strong, and the papaya flavored peanut spread had an undesirable color.

## 3. Chocolate Flavored Peanut Beverage

Chocolate flavored peanut beverage was formulated to contain 3.5% protein from peanut protein isolate, 3.5% fat, 6 to 10% sugar (sucrose), 0.5 to 0.9% cocoa powder, stabilizer, and ingredients with water. The process consisted of the following steps: (1) blending of ingredients with water, (2) warming to 80°C, (3) homogenizing at 2500 psi for 2 min, (4) bottling, (5) pasteurizing at 80°C for 15 min, and (6) refrigerating the product. Three levels of each variable (6, 8 and 10% sugar; 0.5, 0.7 and 0.9% cocoa powder) were chosen to make a total of nine treatment combinations. The nine formulae were prepared and refrigerated before subjecting to sensory evaluation. Eighteen trained panel members were selected from laboratory personnel and students. Panelists were asked to rate samples for chocolate color, peanut odor, sweetness, viscosity and overall acceptability, using a 100 mm unstructured line scale. The results demonstrated that good quality chocolate flavored peanut beverage can be produced which contains 3.5% peanut protein isolate, 3.5% fat, 8% sugar, 0.7% cocoa powder, 0.1% stabilizer and water. The quality of the product was close to that of chocolate milk.

Sensory characteristics were rated acceptable by 96% of the consumer panel members. Results indicate that an acceptable chocolate flavored peanut beverage can be produced using an optimal ratio of sugar and cocoa powder. Larger scale consumer tests are needed to confirm the acceptance of the product. Formulation of a milk-shake type product based on peanut protein isolate may be possible following a similar formulation process. Fruit flavored beverage products may be another avenue for expanding the utilization of peanuts in the form of beverages simulating dairy type products.

## 4. Chicken Patties Extended with Peanut Flour

The objective of this study was to determine the quality attributes of ground chicken extended with defatted (DPF) and partially defatted (PDPF) peanut flours. Defatted and partially defatted peanut flours were steamed for 30 min at 100°C. Flours were then dried in a solar dryer for 24 hr or until the  $a_w$  dropped to 0.50. The flours were ground using a centrifugal mill equipped with a 0.25-mm screen and stored in the refrigerator until used. Peanut flour from each treatment was added to

ground chicken meat, together with other ingredients. Mixing was done by hand. The mixture was refrigerated for 30 min and then molded into 8-cm dia. patties. Individual patties weighed 85 g. Three levels (10, 15 and 20%) of each type of flour were used to prepare the patties. The resulting six treatments were subjected to quality evaluation immediately after cooking by deep frying at 150°C for 5 min, and excess oil was removed.

Results indicate that cooking losses and shrinkage of extended ground chicken patties were less than those of the pure chicken patties control. Extending ground chicken with peanut flour increased the percentage of water retained by patties during cooking. For texture evaluation, the shear forces required to compress extended patties were less than that required for the control. The higher water-retention properties of extended ground chicken patty evidently contributed to their increase in tenderness as indicated by lower compression values. Color scores for extended ground chicken patties with 20% DPF and 10% PDPF were lower than that of the control patties. Higher scores for firmness and flavor were also assigned to the control patties, but the pure chicken patties were less tender than the extended patties. All extended chicken patties had peanut odor, especially those extended with PDPF. There was a slight difference in cohesiveness among the products, but no difference in juiciness was observed. There was also no difference in overall preference scores among the control and the extended patties with 15% and 20% DPF and 10% PDPF.

These results indicated that extended chicken patties could be prepared by using either DPF or PDPF. Extending with PDPF is more appropriate in Thailand because of lower processing costs.

### C. Accomplishments at the University of the Philippines

#### 1. Survey and Sensory Evaluation of Existing Peanut Products

Despite the popularity of peanuts in the Filipino diet, the per capita consumption in the country is low, representing only a minor dietary item. This was confirmed by a nationwide survey of the consumption patterns of raw/processed peanuts and 33 selected food items we conducted in the past year. The average per capita consumption per month of raw peanut was 182.95 g. Among the peanut products, boiled (153.36 g), roasted (111.91 g), fried (111.08 g) and peanut butter (73.20 g) were the top four items with high consumption patterns while peanut oil was relatively unknown to Filipino consumers.

The survey also confirmed the high acceptability for peanuts among Filipino consumers, but the high cost of the commodity may explain its minimal consumption in the Philippines. With the current economic situation, wherein food accounts from 40 to 50% of the household income, buying peanuts is considered a luxury. Peanuts, which are usually processed into snack items and used as a food ingredient were consumed less frequently (weekly or monthly), as compared to other food items (rice/meat/poultry/fish/ vegetables), which were regularly consumed daily or weekly.

Furthermore, the high costs of prime commodities, particularly the traditional sources of protein such as meat and dairy products, resulted to inadequate energy and protein consumption per day as compared to the RDA issued by the Food and Nutrition Research Institute. Thus, there is a need to develop cheaper alternative sources of protein. The potential of peanuts for developing additional food products, utilizing its proteins, lipids, vitamins, minerals and flavor are great. Possibly no other crop has as many advantages as a food ingredient as does peanut.

The development of new products from peanuts as well as the improvement of existing peanut products must be pursued. Very few studies have been done in the Philippines in this area. Quality peanut products will assure consumers of safe and nutritious food. In addition, an increased demand for peanuts will encourage farmers to increase production. In an earlier phase of this project, preliminary findings showed that peanut milk and peanut curd were acceptable to taste panelists.

The start of this present phase of the project includes the cataloging of the existing peanut products available commercially in the Philippines which vary widely in their wholesomeness, quality and shelf-life. These variations are often attributed to raw material quality, processing operations and

storage of the product. Peanut products collected in the market were described and evaluated for sensory qualities. Aflatoxin surveillance of both raw and processed products was done. A compilation of the process for these various products as well as the new products to be developed will be done to facilitate information dissemination. This will provide avenues for setting up small-scale enterprises for generating income and employment.

The peanut products were collected from the 13 regions of the Philippines. Samples were either bought from commercial outlets or from the peanut processors. For some products, processing was documented wherein the process/method was demonstrated by the manufacturers during the survey. The source, region, province, names and addresses of manufacturers were recorded. Sensory evaluation of 15 of the 18 products was done using a 150-mm linear scale. Evaluation was done by 13 experienced panelists, twice for each product. Results of these evaluations are summarized as follows:

a. **Peanut Adobo.** In the Philippines, peanut adobo refers to the deep-fried peanuts (with or without skin) seasoned with salt and garlic or pepper. This is the most popular way of consuming peanuts. Peanut adobo is sold in most towns and cities at street corners near moviehouses and markets. The general acceptability for peanut adobo was high with scores ranging from 80.2 to 116.1 for the two samples from Cebu, three from Baguio and one from Cotabato. In Mindanao (Regions 9 to 12), the addition of pepper to fried peanut was common and the product is called hot or spicy peanut.

b. **Peanut Brittle.** Peanut brittle consists of whole or ground peanuts cooked with sugar. For crispier and better flavor, butter, corn syrup or glucose are added instead of cane sugar. This made peanut brittle from Baguio highly acceptable. Products were lighter in color and had a better appearance than the others evaluated. Baguio City is the most preferred source for this product by consumers.

c. **Peanut Butter.** Peanut butter was the most preferred peanut product by Filipinos. Besides being used as a bread spread, it is also used as an ingredient in cooking various dishes, among which is steak from goats, locally known as kaldereta. The basic steps in peanut butter manufacturing consist of cleaning of shelled peanuts, roasting, blanching, blending of ingredients, grinding, cooking and packaging. Salt, sugar and oil are usually added. There are a large number of brands being sold in the market. Processing ranges from small or home scale to large scale operations. The panelist's were able to detect the superior quality of the two Lady's Choice brands as indicated by the significantly high acceptability scores compared to the other brands. The more acceptable samples were light brown in color. The dark brown color of other products was either due to the use of over roasted peanuts or peanuts with seedcoat. Texture of the preferred products varied from very smooth to grainy or chalky.

Oil separation was another problem encountered by some peanut butter manufacturers, particularly the home processors. This was not evident for some large scale manufacturers because emulsifiers/stabilizers such as hardened vegetable oil are added which make the products four to five times more expensive than the brandless peanut butter sold in the market.

d. **Coated Peanuts.** Coated peanuts are made by cooking a mixture of water, refined sugar and peanuts over a low fire until thick. A good coated peanut is shiny. Six samples of coated peanut were evaluated and general acceptability scores ranging from 72 to 90 were not significantly different. The color of the final product depends on the color of the peanut. Brown and red peanut varieties were most popular.

e. **Greaseless Peanut.** Greaseless peanuts are deskinning peanuts which contain less oil than raw peanuts and are seasoned with salt. Five brands were evaluated. Texture and saltiness scores were not significantly different.

f. **Panutsa.** This is a native candy and is a popular Batangas product. It can also be found in Baguio and Bicol. The product is being marketed in various sizes. Water and crystallized molded cane or brown sugar are cooked to the desired consistency; whole peanuts (with skins) are then com-

bined with the syrup and cooked until the consistency becomes thick. The mixture is then cooled slightly and formed into a desired shape.

Panutsa from Batangas was more acceptable than panutsa from Baguio. The former products also had better appearance and color. Characteristics of the product are largely dependent on type of sugar used and length of cooking.

g. **Pinato.** This peanut product is popular in the Southern part of the Philippines and is probably the counterpart of panutsa in Luzon. The product is sold in various sizes and shapes (round, flat and rectangular, cone shape). Peanuts are roasted and skins are removed. The peanuts and sugar are cooked under low fire and molded while still hot. Other ingredients are also added depending on the locality. These include cooked rice in Davao, linga (sesame seed) in Zamboanga and honey in Camiguin.

h. **Roasted Peanut Without Shell.** Roasted peanuts with or without skins are consumed in the Philippines. Salt, garlic and other seasonings are usually added. Newly harvested locally grown peanuts are preferred for roasting rather than larger imported peanuts because they have better flavor and are sweeter than generally bland imported peanuts.

i. **Roasted Peanut With Shell.** Roasting is usually done in big drums, either manually operated or automated. Of the four samples evaluated, only one was acceptable. Even the well-known commercial brands were unacceptable due to very hard texture. These samples were very dry. No significant differences in saltiness and off-flavor scores were observed.

j. **Peanut Turon.** Peanut turon consists of peanuts mixed with sugar and wrapped in lumpia wrapper before deep frying. Each turon measures around 5 x 1 cm. There were no significant differences for attributes except for sweetness for the three samples evaluated. The generally low acceptability (75.0) could be due to the unfamiliarity of the panelists from Laguna to this product since it is only manufactured and sold in Cebu (Central Visayas), Davao and Cebu (Mindanao).

k. **Turonones de Mani.** The package of this product listed the following ingredients: enriched flour, sugar, vanilla, peanut, butter, powdered milk, corn syrup and eggs. It was individually wrapped measuring about 14 x 1 cm. This is popular in Cebu and Bulacan. A sample from Bulacan was the most acceptable among the five products evaluated, but in general, all were highly acceptable to the panelists.

l. **Peanut Cake.** Peanut cake is a Chinese delicacy. Ingredients consist of peanuts, glucose and sugar; products about 2 x 1.5 x 2 cm in dimension are individually wrapped. There were no significant differences in acceptability, sweetness and texture of the two samples evaluated. Peanut cake was very acceptable, a little bit sweet and brittle (neither soft nor hard).

m-n. **Peanut Broas/Peanut Cookies.** Lola Pureza Bakery in Mandaue, Cebu is the sole manufacturer of peanut broas in the Philippines. It has the following ingredients: 60% peanut, flour, eggs, salt and leavening agent. The finished product is peanut in shape. Peanut cookie is being manufactured by New Echague Food in Quezon City with peanuts, flour, shortening and glucose as ingredients. Both products were highly acceptable, soft and a little bit sweet.

o. **Peanut Pastillas.** Pastillas consist of ground peanuts mixed with flour and sugar and cooked until thick. The product is individually wrapped as about 4 x 0.5 cm pieces. No significant differences were found in acceptability.

p. **Peanut Masareal.** This product can only be found in Cebu (Region 7). Deskinning peanuts are boiled until soft, ground, mixed with sugar and cooked over low fire.

q. **Peanut Kisses.** Another delicacy famous in Bohol (Region 7) is peanut kisses. It is shaped like Hershey's chocolate kisses. The formulation for this product remains unknown to other peanut processors.

r. **Peanut Polvoron.** Polvoron has the following ingredients: flour, skimmilk, ground roasted peanuts, sugar and melted margarine. After mixing all the ingredients, it is molded and wrapped individually. In addition to the eighteen products described above, peanuts are also used as ingredients in cooking. A variety of Filipino dishes such as meats, vegetables, baked items, desserts, native delicacies and pasta contain peanuts. Based on the survey conducted by our laboratory in the thirteen regions of the Philippines regarding food preparations using peanuts, kare-kare, lumpia, cake and kaldereta were the four most commonly prepared dishes which contain peanuts.

## 2. Aflatoxin Surveillance of Commercially Sold and Consumed Peanuts

Realizing the seriousness of aflatoxin contamination and its implications to public health, a total of 58 peanut seed samples and 18 peanut products consisting of 84 samples were analyzed qualitatively for aflatoxin content. Results of the analyses showed that 27.3% of the products and 55.1% of the raw peanuts analyzed were positive for aflatoxin, and contamination occurred in the moisture content range from 6.12% to 12.09%. Most of the contaminated samples had a moisture content well below the critical value of 10% when analyzed. The results suggest that the samples analyzed were probably contaminated before they were dried. Common drying practices used by Filipino peanut farmers are to leave uprooted peanut seeds in the field under the sun for two to three days, or to air-dry by spreading the peanut seeds on the house floor or other places. These drying methods favor mold invasion because of the wet condition in the field, moisture due to fog and dew, unsanitary conditions in the field and the length of drying time.

Other practices which may contribute to aflatoxin production in peanuts are:

- a. The araro (plow) system of harvesting when the soil is arid and hard. This damages the pods during harvesting.
- b. Waiting for the rain to come before harvesting so that pulling the peanuts out of the ground will be easier. When harvested, pods are overmature and in many cases the kernels/seeds are germinated.
- c. Dried peanuts sold to traders are stored for as long as 10 months without controlling environment. This causes deterioration of peanuts which then become more susceptible to aflatoxin invasion.

The best approach to contain aflatoxin is prevention. The best precautionary measure is to prevent the infection of peanuts by *Aspergillus flavus* and *A. parasiticus* in the field and during storage. Control starts from the time of planting through harvesting and storing, and ends only when the peanut is consumed. However, interviews with farmers and consumers revealed that the majority have very little if any knowledge about aflatoxins. Unless they are educated about proper harvesting and post harvest handling technologies, control of aflatoxin is unlikely to occur.

## 3. Microbiological Control of Aflatoxin

One hundred fifty-nine mold isolates from soil, water and air were tested for their ability to inhibit *A. parasiticus*. Water samples were taken from lakes, swamps, stagnant waters and canals while soil samples were taken from peanut gardens/plantations, and from areas wherein growth of different plants were abundant.

Isolation of molds was done by plating 1 ml and 0.1 ml of  $10^{-4}$  and  $10^{-6}$  dilutions of soil and water in Potato Dextrose Agar (PDA) plates acidified with 10% tartaric acid. Molds isolated from air were obtained by exposing PDA plates in air for 15 min. Plates were incubated at 32°C for 5 to 7 days. For purification, molds were repeatedly streaked on non-acidified PDA plates until pure cultures were obtained. Cultures were maintained on PDA slants at room temperature.

Evaluation of antagonistic activities of isolates toward *A. parasiticus* was done. Results showed that of 159 mold isolates, one was capable of inhibiting the growth of *A. parasiticus*. A few isolates



belonging to the *Aspergillus niger* group were capable of outgrowing *A. parasiticus* initially but recovery was apparent after 3 days.

A mold isolated from air and later identified as *Aspergillus fumigatus* yielded encouraging results in that it restricted the growth of *A. parasiticus*. However, a literature review suggests that some strains of *A. fumigatus* produce toxins. Prudence dictates therefore that the use of this mold in this project should not be encouraged. A mold isolate with characteristics typical of *Cladosporium fulvum* is currently being evaluated. It exhibits excellent antagonistic properties against *A. parasiticus* by completely suppressing it. No growth of *A. parasiticus* was evident after extended incubation. Characteristics of this mold are being evaluated.

#### 4. Development and Evaluation of Yuba (Peanut Film)

Experiments were conducted to develop yuba, a protein-lipid film from peanut milk. Full-fat and partially defatted peanuts were used in preparing milk. Deskinning peanuts (500 g) were soaked overnight in water at refrigeration temperature, then drained and weighed to determine the amount of water absorbed. Water was added to make up a 1:6 (peanut:water) ratio, and the mixture was ground in a blender for 5 min and filtered through a cheesecloth. The procedure was repeated to produce milk from 1:7, 1:8 and 1:9 (peanut:water) ratios.

Partial defatting was done by extraction of ground, deskinning peanuts six times with hexane (1:2, peanut:hexane). Desolventizing was carried out in a hood overnight. The defatted peanut was used to prepare milk from 1:8 and 1:10 peanut:water ratios. Glycerol was added at a 2.5% level to milk before processing. Milks from a 1:6 full-fat peanut:water ratio containing 0, 1.0 and 2.5% glycerol were also prepared. The milk was boiled, then processed at 75 to 85°C to facilitate film formation. Film was harvested periodically, when their mechanical strength so warranted, hung, air-dried and prepared for subsequent analyses. For films produced from full-fat peanuts, expressed oil was blotted using a cheesecloth before analysis. Films were finely ground, screened using a 20-mesh sieve and analyzed for moisture (oven-drying at 100°C), crude protein (micro-Kjeldahl) and crude fat (Soxhlet method - extraction for 16 h with petroleum ether). Carbohydrate content was calculated by difference. Solubles were determined by suspending a 1-g sample of ground film in exactly 35 ml of water in a 125-ml Erlenmeyer flask. The suspension was placed for 1 h in a water bath with shaker at the desired temperature, and then transferred to tubes and centrifuged for 20 min at 1800 rpm. The supernate was withdrawn and total solubles were determined by direct evaporation to dryness and weighing on an analytical balance.

Hardness and breaking strength of film were determined simultaneously using a Neo-Curd meter. Film strips were fastened securely across the mouth of a beaker and a 2.0 mm sensing rod propelled by a 200 g precision spring was used as a probe. The Neo-Curd meter was used to determine the dry/wet tensile strength of films prepared from full-fat milk. Film strips (0.30 cm x 10 cm) were held at one end by a fixed clamp and the other end by a movable clamp attached to a 100 g precision spring. The clamps were then drawn apart until the strip broke. Similar testing conditions were used to determine wet tensile strength, except samples were immersed in water for 30 sec before testing.

For films from partially defatted milk (10 cm x 1.0 cm), strips were tested using an Instron Universal Testing Machine, model 1140, fitted with a load cell of 5.0 kg minimum range and operated using a 50 mm/min crosshead speed and 200 mm/min chart speed. Analysis of burst strength was conducted using a Mullan Burst Machine. Whole films were subjected to an increasing pressure by a rubber diaphragm which was expanded by hydraulic means until the test specimen ruptured. The gage reading at that point was recorded as the burst strength. Films were analyzed by scanning electron microscopy. Films were mounted on a copper strip and coated with gold. The specimens were observed with scanning electron microscope and photomicrographs of the cross sections and surface structure were taken.

Among the four full-fat formulations tested, the highest yield was obtained from milk prepared from 1:8 peanut:water ratio, and a corresponding decrease was observed as the amount of water was decreased proportionately. In the most diluted formulation, film formation occurred only after con-

centration from prolonged heating which resulted to a very low yield. In contrast, partially defatted peanut milk produced films immediately after the processing temperature was reached. This would suggest the possibility of processing partially defatted milk of higher dilution than the formulations tested to obtain a higher yield of yuba.

Chemical analyses revealed that the composition of films is not a function of the amount of water used in the formulations of milk. For successively withdrawn full-fat films (Table 20), a general trend obtained was the increase in protein content and decrease in fat content. Theoretically, capillary active substances such as protein and oil, having a lower specific gravity than water, should decrease. These were effectively incorporated into the films at the earlier stage of production and were almost depleted at the latter part. But the ability of the first batch of films to retain excess (unreacted) oil owing to a thicker and less smooth surface than the last batch of films may account for the observed trend. The increasing efficiency of unreacted oil removal as processing proceeds is reflected in the total chemical composition of films.

The chemical analysis of films from partially defatted peanuts gave a 1:6 fat:protein ratio compared with 2:1 ratio in full-fat films. This indicates the possibility of producing more stable films, in terms of rancidity, from a completely defatted peanut milk. Determination of solubles revealed that partially defatted films have more water solubles than full-fat films which may lead to the conclusion that these are of low associative bonding or lower tensile strength. The excess oil, which accounts for a substantial portion of the films analyzed, plus the much slower rate of film drying are responsible for the determined low soluble percentage in full-fat films.

Determination of strength properties revealed that an increase in glycerol level in milk resulted to a corresponding decrease in the hardness and bitterness and an increase in breaking strength of films. In contrast, an increase in the amount of water in the milk formulation result in a decreased in the wet/dry tensile strength for both full-fat and partially defatted milk. Bursting strength tests showed no definite trend as affected by the water level in milk owing to the variability of test specimen used. In general, partially defatted films were stronger than full-fat films. This observation was supported by examination of films using a scanning electron microscopy. Partially defatted films have a generally more compact structure than full-fat films which appeared porous.

## 5. Studies on Peanut Nougat

Problems encountered in the preparation of peanut nougat concerned the color and texture. Initially, the color of the experimental nougat produced was darker than commercially available nougat, which is creamy white in color. This problem was corrected by the addition of the cream of tartar in the formulation which helped produce a vastly improved end-product in terms of color. However, the effect of this formulation modification on the product was reversed by a procedural modification done after another problem concerning the texture was discovered. It was observed that the bite property of nougat, which is initially sticky, became short upon storage. This phenomenon may be due to an incomplete dissolution of sugar in the syrup, which crystallizes during storage. The corrective measure employed was to increase the heating temperature of the syrup which brought about a browning reaction of sugar, thus, adversely affecting the color of nougat. Further modification of the procedure to produce nougat with better color and texture is still necessary in order to be at par with or even exceed the quality of the commercially available nougat.

## 6. Studies on Peanut Sauce

A peanut sauce formulation was found to be extremely acceptable and was ideal for vegetable dishes. However, since it was modeled after a typical Indonesian sauce (satay), some Filipinos may find it too spicy for their taste. Hence, it is important to slightly modify the formulation to produce a sauce which utilizes locally available substitute ingredients and which is more suitable to the Filipino palate.

## TRAINING OUTPUTS

In the area of training, Bernardita L. Santos, a Filipino student, completed requirements for her M.S. degree at UGA. Two other students, Chan Lee (Ph.D. candidate) and Nak Kyung Kim (M.S. candidate) are making substantial progress toward finishing their programs.

Ms. Bernardita Santos spent 4 weeks at UPLB in July, 1987, to work with UPLB project investigators to conduct a survey testing an imitation cheese flavored peanut spread developed at UGA.

A workshop on "Peanut Utilization" was organized by Drs. Larry Beuchat and Tommy Nakayama and held at the 7th World Congress on Food Science and Technology, September 28 through October 2, 1987 in Singapore. Included among six speakers from six different countries were Dr. Vichai Haruthaithanasan (KU), Dr. Virgilio Garcia (UPLB) and Dr. Robert Brackett (UGA). Proceedings from the workshop will be published by Congress organizers. Other project investigators attending the Congress were Drs. Anna Resurreccion (UGA), Chintana Oupadissakoon and Penkwan Chompreeda (KU), and Ricardo del Rosario and Reynaldo Mabesa (UPLB).

## TRAVEL

Dr. Brackett visited KU in September, 1987 and Dr. Resurreccion visited UPLB in October, 1987. Drs. Garcia, del Rosario and Mabesa visited KU in October, 1987. Dr. Beuchat visited KU and UPLB in April, 1988. Dr. del Rosario visited UGA in June, 1988. Lengthy discussions concerning project activities and directions as well as field visits took place at the Singapore Congress and at visitations at UGA, KU and UPLB. In addition to visiting UGA, Dr. del Rosario also attended the Basic Symposium and Annual Meeting of the Institute of Food Technologists in New Orleans.

Visitations at professional meetings and at investigators' laboratories enhanced professional development. Interaction affords investigators opportunities to share project information in a timely manner and to more effectively administer research activities in respective laboratories.

## PUBLICATIONS

## A. Scientific Journal Articles

- Chompreeda, P., A. V. A. Resurreccion, Y.-C. Hung and L. R. Beuchat. 1987. Quality evaluation of peanut-supplemented Chinese-type noodles. *J. Food Sci.* 52:1740-1741.
- Chompreeda, P., A. V. A. Resurreccion, Y.-C. Hung and L. R. Beuchat. 1988. Modeling the effect of peanut and cowpea flour supplementation on quality of chinese-type noodles. *Intl. J. Food Sci. Technol.* (Accepted)
- Chompreeda, P., A. V. A. Resurreccion, R. D. Phillips and L. R. Beuchat. 1988. Nutritional quality of noodles supplemented with peanut and cowpea flours. *J. Food Sci.* (In preparation)
- Hao, Y.-Y., and R. E. Brackett. 1988. Removal of aflatoxin B1 from peanut milk inoculated with *Flavobacterium aurantiacum*. *J. Food Sci.* (Accepted)
- Kim, N. K., and Y.-C. Hung. 1988. The effects of harvest time and maturity on the chemical composition and mechanical properties of peanut. *J. Food Sci.* (In preparation)
- Muego, K. F., A. V. A. Resurreccion and Y.-C. Hung. 1988. Characterization of texture of spreadable peanut products by instrumental and sensory methods. *J. Texture Studies.* (In preparation)
- Resurreccion, A. V. A. 1988. Comparison of sensory quality of spreadable peanut products. *J. Food Sci.* (Accepted)

- Resurreccion, A. V. A., and J. B. Blackburn. 1988. Relationship between sensory quality and flavor volatiles in headspace gas of spreadable peanut products. *J. Food Sci.* (In preparation)
- Rubico, S. M., R. D. Phillips, A. V. A. Resurreccion and L. R. Beuchat. 1988. Nutritional, microbiological and sensory quality of peanut beverage prepared under different processing conditions. *J. Food Sci.* (In preparation)
- Rubico, S. M., A. V. A. Resurreccion and L. R. Beuchat. 1988. Comparison of sensory properties and headspace volatile of a peanut beverage processed at different temperature and time conditions. *J. Food Sci.* 53:176-180.
- Rubico, S. M., A. V. A. Resurreccion, J. F. Frank and L. R. Beuchat. 1987. Suspension stability, texture and color of high temperature treated peanut beverage. *J. Food Sci.* 52:1676-1679.
- Santos, B. L., and A. V. A. Resurreccion. 1988. Effect of particle size reduction on the quality of peanut pastes. *J. Food Qual.* (Submitted)
- Santos, B. L., A. V. A. Resurreccion and V. V. Garcia. 1988. Consumer acceptance of a peanut-based Imitation cheese spread. *J. Food Science.* (Accepted)
- Sukhumsuvun, S., and A. V. A. Resurreccion. 1988. Food habits and eating patterns of Thai nationals in the United States. *Nutr. Rept. Intl.* 37:913-922.
- Sukhumsuvun, S., and A. V. A. Resurreccion. 1988. Perceptions of peanuts and related products by Thai consumers in the U. S. *Intl. J. Food Sci. Technol.* (Accepted)
- Sukhumsuvun, S., A. V. A. Resurreccion and L. R. Beuchat. 1988. Development and optimization of a spread made from peanut tofu. *J. Food Sci.* (In preparation)

#### B. Miscellaneous Technical Publications

- Brackett, R. E. 1988. Strategies for dealing with aflatoxins in peanuts. *Proc. 7th World Congress of Food Science and Technology.* 28 Sept. - 2 Oct., 1987, Singapore (Accepted).
- Garcia, V. V. 1988. Overview of peanut utilization in the Philippines. *Proc. 7th World Congress of Food Science and Technology.* 28 Sept. - 2 Oct., 1987, Singapore (Accepted).
- Haruthaithanasan, V. 1988. Overview of peanut utilization in Thailand. *Proc. 7th World Congress of Food Science and Technology.* 28 Sept. - 2 Oct., 1987, Singapore (Accepted).
- Oupadissakoon, C., V. Haruthaithanasan and P. Chompreeda. 1987. Utilization. *In Thailand Coordinated Groundnut Improvement Program Under Collaboration with the Peanut Collaborative Research Support Program (Peanut CRSP).* Progress Report for 1987. Dept. of Agric., Min. of Agric. and Coop., Khon Kaen Univ. and Kasetsart Univ. pp. 85-95.
- Santos, B. L. 1987. Development of a peanut-based imitation cheese spread product. Master of Science Thesis. University of Georgia, Athens. 88 p.
- Santos, B. L., and A. V. A. Resurreccion. 1988. Sensory quality, texture and color of paste prepared from peanut kernels. *Proc. 7th World Congress of Food Science and Technology.* 28 Sept. - 2 Oct., 1987, Singapore (Accepted).

#### C. Abstracts (of PRESENTATIONS)

- Chiou, R. Y. Y. 1988. An indigenous view of peanut utilization in Taiwan. *Prog. 7th Congress of Food Science and Technology,* 28 Sept. - 2 Oct., Singapore. p. 19.

- Chompreeda, P., A. V. A. Resurreccion, Y.-C. Hung and L. R. Beuchat. 1987. Modelling the quality of chinese type noodles supplemented with peanut and cowpea flours. Prog. 7th World Congress of Food Science and Technology, 28 Sept. - 2 Oct., Singapore. p. 20.
- Kim, N. K., and Y.-C. Hung. 1988. Effect of maturity and harvest time on the mechanical properties of peanuts. Prog. Annu. Mtg. Inst. Food Technol., 19-22 June, New Orleans. p. 189.
- Muego, K. F., A. V. A. Resurreccion and Y.-C. Hung. 1988. Characterization of the textural properties of spreadable peanut based products. Prog. Ann. Mtg. Inst. Food Technol., 19-22 June, New Orleans, LA. p. 202.
- Muego, K. F., A. V. A. Resurreccion and Y.-C. Hung. 1988. Evaluation of the textural properties of peanut butter and peanut paste using instrumental methods. Prog. Annu. Mtg. Food Sci. Human Nutr. Sec., Southern Assn. Agric. Sci., 31 Jan. - 3 Feb., New Orleans, LA. p. 4.
- Resurreccion, A. V. A. 1988. Comparison of flavor quality of peanut based pastes and peanut butter by sensory methods. Prog. Annu. Mtg. Food Sci. Human Nutr. Sec., Southern Assn. Agric. Sci., 31 Jan. - 3 Feb., New Orleans, LA. p. 16.
- Resurreccion, A. V. A., and B. L. Santos. 1988. Sensory quality, texture and color of pastes prepared from peanut kernels. Proc. Georgia Nutr. Council Mtg. Res. Conf., 25-26 Feb., Pine Mountain, GA. p. 24.
- Santos, B. L., and A. V. A. Resurreccion. 1987. Comparison of processing methods for the preparation of a bland peanut paste. Annu. Mtg., Amer. Peanut Res. Educ. Soc., 14-17 July, Orlando, FL. p. 120.
- Santos, B. L., A. V. A. Resurreccion and V. V. Garcia. 1988. Development and quality evaluation of a peanut-based imitation cheese spread. Prog. Annu. Mtg. Inst. Food Technol., 19-22 June, New Orleans, LA. p. 229.
- Santos, B. L., A. V. A. Resurreccion and P. E. Koehler. 1988. Development of a peanut based imitation cheese spread. Proc. Georgia Nutr. Council Ann. Mtg. Res. Conf., 25-26 Feb., Pine Mountain, GA. p. 24.
- Wirakartakusumah, M. A. 1988. Present status of peanut utilization in Indonesia. Prog. 7th World Congress of Food Science and Technology, 28 Sept. - 2 Oct., Singapore. p. 101.
- Zain, A. M. and M. N. Azudin. 1987. Status of production and utilization of peanut in Malaysia. Prog. 7th World Congress of Food Science and Technology, 28 Sept. - 2 Oct., Singapore. p. 105.

#### PLANS FOR 1988-89

1. Continue work to optimize processing conditions for producing high quality peanut pastes and beverages.
2. Survey Thai consumers to determine their attitudes and perceptions toward selected peanuts and peanut products.
3. Optimize formula and process for a peanut-supplemented confectionery product acceptable to Thai consumers.
4. Determine Thai consumer acceptability of peanut spread and peanut-supplemented sausage and noodles.
5. Investigate the inhibitory activity of a mold isolate on growth of *Aspergillus parasiticus*.
6. Evaluate storage stability of peanut films made from full-fat and partially defatted peanuts.

7. Continue work on fermentation of ammoniated, aflatoxin-contaminated peanuts/peanut meal.
8. Determine feasibility of using sodium bisulfite, ultraviolet light and density segregation for reducing aflatoxin levels in peanuts.
9. Continue work to develop various peanut-based products, including nougat, peanut sauce, peanut cake, turones de mani and other products having high potential for acceptance.
10. Investigate fermentation characteristics of lactic acid bacteria in peanut milk in an effort to develop substitutes for fermented dairy products.
11. Initiate discussions and plans for transfer of handling/drying/storage technologies developed in the GA/FS/CAR project, "Farming Systems for the Small Peanut Producer," to the Philippines and Thailand.

AAMU/FT/S  
**An Interdisciplinary Approach to Optimum  
 Food Utility of the  
 Peanut in SAT Africa**

Alabama A & M University—Burkina Faso  
 Bharat Singh, Principal Investigator

**Introduction:** This project was designed to address the constraints which limit the maximum utilization of peanuts for human consumption in SAT Africa. In the first 5 year period of the project, data were collected to determine variations in environment, socioeconomics and food technologies as they constrain the preservation and utilization of peanut and peanut products in one of the major peanut producing countries in SAT Africa, namely, Sudan. Based on consumption and postharvest surveys in three distinct regions of Sudan, it was recognized that peanut utilization could be considerably increased if efforts were made: (a) To increase utilization of peanut into more refined / processed forms. (b) To improve packaging of peanut and peanut products to increase the shelf-life. (c) To utilize peanut flour (after extraction of oil) to increase protein value of cereal-based foods. (d) To improve the method of storage, post-harvest handling and inventory management. Recent observations made in other SAT countries including Burkina Faso, Senegal, Niger and Mali indicate the existence of almost similar problem in peanut utilization. The main focus will be to conduct collaborative studies with scientists in peanut production and utilization: (a) To develop cultivars with acceptable quality for processing and utilization. (b) To maintain quality of peanut during postharvest handling including freedom from insect damage and mycotoxin contamination. (c) To increase utilization of peanut products in enhancing the protein levels of cereal-based indigenous food items.

**Major accomplishments.**

- (1) An MOU between Alabama A & M University and the University of Ougadougou in Burkina Faso has been completed and research has been started at the University of Ougadougou on evaluation of quality of peanut during storage.
- (2) A study on fortification of a sorghum-based food product, "kisra" has been completed. Results indicated that acceptable kisra of nutritionally superior quality could be prepared from sorghum flours with 30% defatted peanut flour. Addition of defatted peanut flour resulted in improvement of baking-ease, color and texture of the final product. In addition, defatted flour enhanced the nutritive value as evidenced by increased protein, lysine, methionine and other essential amino acids and improved the protein digestibility of the product. As a result of these enrichments, the ratio of leucine/isoleucine and leucine/lysine in sorghum-peanut composite flours has been altered favorably, hence diminishing the pellagragenic characteristic of food products derived from the straight sorghum flour.
- (3) Studies on effects of water- and steam-blanching and packaging materials on shelf-life, sensory qualities and nutrient composition of peanuts have been completed. Blanching by water or steam and using glass jars as packaging materials improved the stability and extended the shelf-life of peanuts up to three (3) months as evidenced by lower peroxide value. Steam-blanching was more effective in prolonging the shelf-life of salted roasted peanuts than water-blanching. Sensory evaluation data indicated that the freshly roasted unblanched peanuts were more preferred than the blanched roasted peanuts for texture, flavor and color. However, sensory evaluations of the stored roasted peanuts showed that the flavor scores of unblanched roasted peanuts decreased with storage time while the flavor scores of the blanched roasted peanuts were stable throughout the storage period.
- (4) A study has been completed on development of a method to prepare a spiced yogurt-like product called "mish" from the peanut milk. Usually "mish" is prepared from whole milk. However, results of this study indicated that acceptable mish could be prepared from peanut milk. The consistency, flavor and texture of this product were found to be similar to the traditional mish prepared from the whole milk.
- (5) A study involving additions of partially defatted peanut flour in the formulation of sugar snap cookies replacing 20% of the normal wheat flour has been completed. Within the study design various preparations of faba bean flours were included in cookie formulations, and sensory evaluations of the several experimental materials were contrasted to full wheat flour preparations. It was clear that the flavor of peanut was discernable in the cookies formulated

with the peanut material and was no more acceptable than several of the faba bean formulated cookies and discernably less acceptable than the full wheat formulated product.

- (6) Accomplishments through studies of alternate procedures to roast confectionary peanuts as are deemed appropriate for the SAT Africa region include: a) An alternate method employing ceramic beads as a particle-bed heat transfer medium (in contrast to traditional efforts with sand as the heat transfer medium) was shown to produce acceptable product quality characteristics to the same degree as dry roasted processing employing an air media within a cabinet (tray) convection roaster. b) Of two bead sizes tried for the ceramic heat transfer media, the larger (>2.45 mm) were judged to be equally acceptable in terms of confectionary qualities of the nuts by sensory perceptions as those roasted in the smaller beads of approximately 1.53 to 2.45 mm diameters. c) Confinement of the ceramic media in a deep, cast-iron frying pan or alternately in a wok pan were contrasted with better results coming in the frying pan as evidenced by less charring of the product because of a presumably more uniform heat distribution in the former with more surface contacting the heat source.
- (7) Peanut paste stability was studied by comparing the factors of preparation and storage in 2<sup>(5-1)</sup> half-fractional factorial design. It became clear that stability as monitored by peroxide values of the stored peanut pastes was not greatly influenced by milling method whether with traditional stone grinding processes or by an effort with a manually operated plate mill or by additions or deletions of chelator in the form of EDTA. Instead, the most outstanding effect was temperature of storage followed by the effect of storage in the presence of fluorescent lighting interacting with temperature (a negative interaction) and the effect of storage in the presence of air interacting with temperature (a positive interaction).
- (8) Scientists at the University of Ougadougou have completed analyses of peanuts grown in the year 1987-1988. The data include determinations of protein, fat, vitamins and aflatoxins.
- (9) Two Sudanese students have completed their Master of Science degrees in Food Science at Alabama A&M University.
- (10) An MOU was completed in January 1988 and research has since begun at the University of Ougadougou.

#### **Expected impacts of the project.**

The major constraint in increasing peanut utilization in SAT Africa is the unavailability of peanut in processed forms: packaged roasted peanuts, peanut butter, defatted peanut flour, peanut milk, candies and other confectioneries at affordable costs. Secondly, contamination of peanut with molds, insects, and mycotoxins further limits the utility for human consumption. The project aims to establish research at the University of Ougadougou to address these constraints. It is expected that research results will lead to better utilization of peanuts in SAT Africa. Specifically, the research results will lead to improvement of methods of storage and packaging, processing and development of a variety of products. Further, the objectives of the projects include collaborative research with plant scientists, microbiologists and entomologists to improve the quality of peanut available for human consumption.

#### **Goals**

##### **General Goal**

To foster interdisciplinary (nutrition, food science, social and economic) institution-based linkages between U.S. and LDC scientists serving major peanut producing and consuming populations of the Semi-Arid Tropic regions of Africa for the purpose of optimizing the food utility of the peanut.

##### **Specific Goals**

Specific goals of the project are consistent with the general goal of the peanut CRSP to develop collaborative research and development programs on the peanut between scientists at Alabama A & M University and the University of Ougadougou in Burkina Faso.



**ORGANIZATION****USA****Alabama A & M University**

Dr. Bharat Singh, Principal Investigator, Department of Food Science, Normal, Food Scientist.  
 Dr. John C. Anderson, Cooperator, Department of Food Science, Normal, Food Scientist.  
 Dr. D. R. Rao, Cooperator, Department of Food Science, Normal, Nutritionist.

**Burkina Faso****University of Ougadougou**

Dr. Alfred S. Traore, Principal Investigator, Food Scientist.  
 Mr. Solibo Some, Entomologist, Cooperator.  
 Mr. Albert Ouedraogo, Entomologist, Cooperator.  
 Mrs. Lactitia Ouedraogo, Food Technologist, Cooperator.  
 Dr. Alain N. Sawadogo, Director, I.S.N., Cooperator.  
 Mr. Herve Campaore, Food Technologist, Cooperator.  
 Mr. Francois T. Ouedraogo, Food Technologist, Cooperator.  
 Dr. Philippe Sankara, Phytopathologist, Cooperator.

**Graduate students and research projects for thesis**

1. Dike Ukuku: Determination of soluble sugars, volatile fatty acids, amino acids and organic acids in leachates from water and steam blanching of peanuts.
2. John U. Anyanwu: Processing and storage stability studies on "Kuli-kuli".
3. Abraham Idowu: Incorporation of peanut flour as alternate adjunct in studies of composite flour in sugar snap cookies (graduated).
4. Francis Agbo: Studies of stability of peanut pastes through factorial storage variations and stone versus plate milling processes.
5. Isameldin Hashim: Effects of blanching and packaging materials on sensory quality and stability of salted and unsalted roasted peanuts (graduated).
6. Ahmed Murtada Ahmed: Nutritional improvement of sorghum based kiswa using defatted peanut flour (graduated).
7. Mike Ogwal: Studies of visco-elastic characters of peanut butters by variations of formulation and homogenization processes.

**Accomplishments in Detail**

**EFFECTS OF BLANCHING AND PACKAGING MATERIALS ON THE SENSORY QUALITY AND STABILITY OF SALTED AND UNSALTED ROASTED PEANUTS (I. B. Hashim and B. Singh)**

Storage stability of salted roasted peanuts at room temperature is about three weeks. The purpose of roasting peanuts is to promote flavor changes which increase the overall palatability of the nuts. Consumption of peanuts as nuts and in the manufacture of peanut butter and other products has been based on the use of roasted peanut seed. The term "blanching" in peanut processing means removal of the testa or the seed coats from peanuts. Several procedures have been developed for blanching peanuts, e.g., dry, water, spin, steam, alkali, and hydrogen peroxide blanching. Blanching provides a method for upgrading the peanuts that failed to meet the grade standards. Lipid oxidation and rancidity in peanuts may involve enzyme catalysis. Lipoxigenase, the enzyme responsible for oxidation at double bonds of unsaturated fatty acids, is found to be totally inactivated in peanuts treated with hot water at 86°C for 90 sec or steamed for two minutes at 100°C, and activity is reduced 50% by immersion of the peanuts in hot water at 79°C for 90 sec. Water-blanching roasted peanuts have a longer shelflife at room temperature compared to unblanched and spin-blanching roasted peanuts.

The purposes of this study were: 1. To investigate the effect of water and steam-blanching and salting on the sensory quality of fresh roasted peanuts and 2. To determine the effects of packaging materials combined with blanching and salting on the sensory quality and stability of roasted peanuts stored at room temperature for six months.

For this study, Florunner US No.1 peanuts were purchased from Gold Kist (Atlanta, GA) and divided into three batches: The first batch of peanuts was unblanched, the second batch was blanched by dipping the kernels in hot water at 86°C for two min, and the third batch was steam-blanched at 100°C for two min. The blanched batches were dried at 50°C for six hrs to reduce the moisture content. Each batch was divided into two parts. To one part, salt (Leslie Popcorn Salt, Newark, CA) was added at the level of 1% with 0.5% vegetable oil before roasting, and the other part was roasted without addition of the salt. Roasting was done at 163°C for 8 min in a cabinet drier (Proctor and Schwartz, Inc., Horsham, PA). The nuts were cooled to room temperature and skinned manually. Then half of each treatment were packed into polyethylene bags (Ziploc freezer bag, The Dow Chemical Co., Indianapolis, IN) which in turn were packed into black bags, and the other half were packed into glass jars (regular pint Ball Mason, Ball Corporation, Muncie, IN), which in turn were packed into cartons and held at room temperature for up to six months until used for evaluation.

Proximate analyses for raw and roasted peanuts were done using AOAC Methods (1984). Peroxide values and free fatty acid numbers of extracted oil (petroleum ether extraction for eight hours) were determined in duplicate at 0, 1, 2, 3, 4, 5, and 6 months of storage. Peroxide values were determined using AOAC Methods (1978) and expressed as meq peroxide/kg of oil. Free fatty acid numbers were determined using APRES Quality Methods (1983) and expressed as oleic acid %. A consumer panel consisting of 30 to 55 untrained panelists was used for sensory evaluation. The roasted peanut samples were evaluated for preference of texture, flavor and color by using a 6-point hedonic scale to describe the peanuts: with one (1) indicating "dislike very much" and six (6) indicating "like very much". The scale on the score sheets contained alternatives only for ease of choice. The sensory panel was composed of faculty, staff and students from the campus of Alabama A&M University. Sensory analyses were carried out in the sensory evaluation laboratory of the Department of Food Science at Alabama A&M University. Samples for texture and flavor were presented in souffle cups (Huntsville Paper Co., Huntsville, AL) under red light to eliminate color biases. Samples for the color were presented in small bowls under white fluorescent light. Sensory evaluation was done for the fresh roasted peanuts and the stored roasted peanuts each month over a six-month storage period.

Color was measured by using APRES Quality Methods (1983) over a six-month storage period. Hunter-L values (degree of lightness) were determined with a Gardner XL-800 series Tristimulus colorimeter (Pacific Scientific, Gardner Laboratory Division, Bethesda, MD) standardized to a white standard plate.

Texture was measured using Model TP-4C Texture Press with model TRI integrating Texturecorder (Food Technology Corporation, Rockville, MD) over a six-month storage period. Twenty-gram samples of each roasted peanut product were used to measure the texture using standard shear-compression cell. Statistical analyses were performed using the Statistical Analysis System (SAS, 1985) on the IBM mainframe computer at Alabama A&M University. Data were analyzed using the General Linear Model Procedure. Analysis of variance followed Duncan's Multiple Range Test, regression analysis and correlation.

Roasting reduced the moisture content which apparently resulted in increased protein, fat and ash contents (Fig. 1). The decrease in fiber content was due to removal of the seed coats from the roasted peanuts. Means from the sensory evaluation of the freshly roasted peanuts (Table 1) showed that the unblanched roasted peanuts were more preferred than the blanched roasted peanuts for texture, flavor and color. The water-blanched roasted peanuts were more preferred than the steam-blanched roasted peanuts for color. Blanched salted roasted peanuts were preferred more than the blanched unsalted roasted peanuts for texture, flavor and color. The results showed that the blanching affected the sensory attributes significantly, especially the color, but the addition of salt improved the texture and the flavor of the blanched treatments as indicated by sensory evaluation scores.

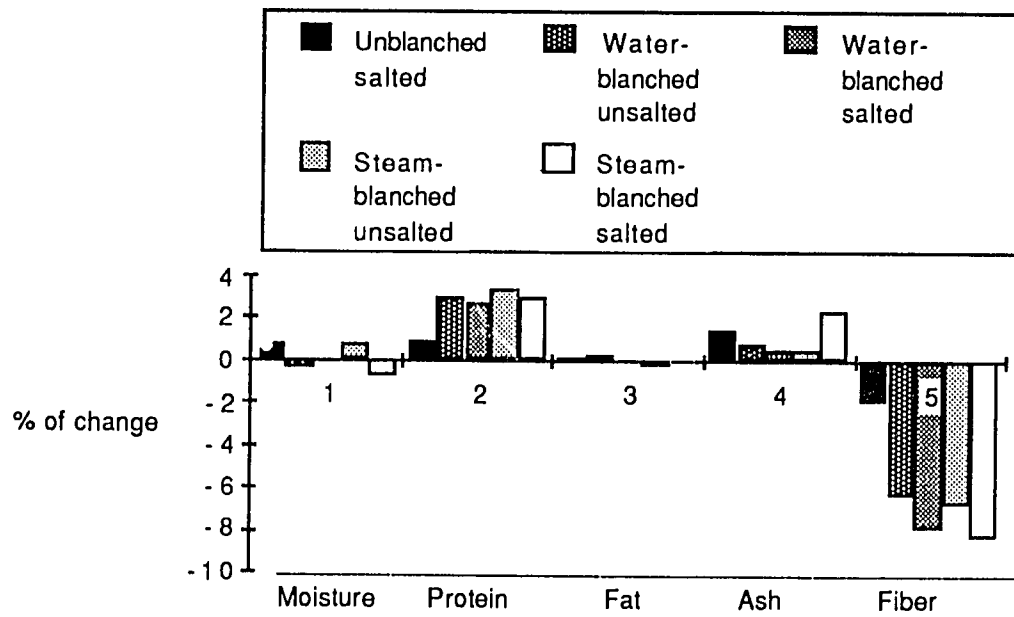


Figure 1. Percentage changes over unblanched unsalted roasted peanuts in moisture, protein, fat, ash and fibre contents of water- or steam-blanched salted or salted roasted peanuts

**Table 1**  
**Effects of blanching and salting on Sensory Parameters of**  
**freshly roasted peanuts<sup>1</sup>**

<b>Treatments</b>	<b>Texture</b>	<b>Flavor</b>	<b>Color</b>
Unblanched unsalted	4.96 a <sup>2</sup>	4.67 a	5.27 a
Unblanched salted	4.91 a	4.76 a	5.18 a
Water-blanched unsalted	4.49 b	4.22 b	4.42 b
Water-blanched salted	4.71 b	4.45 ab	4.60 b
Steam-blanched unsalted	4.49 b	4.20 b	3.89 c
Steam-blanched salted	4.53 b	4.36 ab	3.95 c

<sup>1</sup> Values reported are means of 55 panelist employing 6-point hedonic scale with  
1= Dislike very much to 6= Like very much.

<sup>2</sup> Means for any parameter with the same letter are not significantly different by  
Duncan's Multiple Range Test with alpha = 0.05 probability

Peanuts readily undergo lipid oxidation (oxidative rancidity) because of their high polyunsaturated fatty acids content. The statistical analyses showed there was a highly significant difference due to the storage time which accounted for 91% of the total variation ( $r^2 = .91$ ; Table 2). There were significant interactions between treatments and storage time and between packaging materials and storage time. But it only accounted for 1% of the total variation for each interaction. The results of the analyses were correlated into a multiple-regression formula to predict the peroxide value of the stored roasted peanut treatments based on the storage time. The regression equation found for this relationship was: Peroxide Value (predicted) =  $8.57 + 2.71*(\text{storage time}) + 1.2*(\text{storage time})^2$  for  $r^2 = 0.91$  (Fig. 2). Peroxide value increased with the storage time for all the treatments (Fig. 3).

There were significant differences between the roasted peanuts stored in the polyethylene bags and the roasted peanuts stored in the glass jars (Fig. 4 and 5). Roasted peanuts packed in glass jars were more stable at room temperature than peanuts packed in polyethylene bags for all the treatments as indicated by the peroxide values. The difference between the peanuts packed in the glass jars and the peanuts packed in the polyethylene bags was possibly due to the oxygen permeation through the polyethylene film (420cc/mil/100in<sup>2</sup>/24hr at 73°F and 1 atm). Means for peroxide values of the roasted peanuts stored at room temperature for six months (Table 3) showed there were no significant differences between the salted and the unsalted peanuts for all the methods of blanching. The blanched roasted peanuts were more stable than the unblanched roasted peanuts. The steam-blanched roasted peanuts were more stable than the water-blanched roasted peanuts. Fat is considered rancid if the peroxide value is greater than 20 meq/kg of oil. Therefore, the unblanched salted or unsalted roasted peanuts when packed at room temperature in polyethylene bags were stable for only one month; they were stable for up to two months when packed in glass jars. The blanched (water-blanched or steam-blanched) roasted peanuts were stable for two months when packed in polyethylene bags and up to three months when packed in glass jars at room temperature (Fig. 4 and 5). Apparently, blanching and using the glass jars as packaging material improved the stability of the roasted peanuts as indicated by the lower peroxide values. Steam-blanching was more effective for prolonging the shelf-life of the roasted peanuts.

Means of free fatty acid numbers for the roasted peanuts packed in polyethylene bags or glass jars and stored at room temperature for six months (Table 4) showed that peanuts packed in polyethylene bags or peanuts packed in glass jars showed the same free fatty acids numbers throughout the storage. There were no significant differences among the treatments, although the unblanched peanuts had higher values of the free fatty acid numbers than the blanched peanuts. This may be due to inactivation of lipase enzymes during the roasting for the unblanched peanuts.

The influence of storage on the sensory parameters (Figs. 4 and 5) showed there were slight changes in texture and color with storage time for each treatment. The effects of storage on the flavor of the roasted peanuts (Fig. 6) indicated that the flavor scores of the unblanched peanuts decreased the storage time, but the flavor scores of the blanched peanuts were relatively constant during storage. Peanuts packed in glass jars were more preferred for texture and flavor than peanuts packed in polyethylene bags. Means of sensory parameters for the stored roasted treatments (Table 5) showed there were no differences between salted and unsalted peanuts for each method of blanching for texture, color, and flavor except for the flavor of steam-blanched peanuts. For texture and color the unblanched peanuts were more preferred than the blanched peanuts, but there were no differences between water-blanched peanuts and steam-blanched peanuts. For the flavor, the blanched peanuts were more preferred than the unblanched peanuts. Also, the steam-blanched salted peanuts were more preferred than the steam-blanched unsalted peanuts and the water-blanched (salted or unsalted) peanuts.

The effect of storage time in the degree of lightness ("L") of the roasted peanuts (Fig. 7) showed that there were no changes in the degree of lightness with the storage time for all treatments. There was a difference between peanuts packed in glass jars and peanuts packed in polyethylene bags for the degree of lightness. There were significant differences among the treatments (Table 4); the blanched peanuts were darker than the unblanched peanuts and the steam-blanched peanuts were darker than the water-blanched peanuts as indicated by the lower values of "L".

**Table 2**

Analysis of variance for peroxide values of roasted peanuts stored for six months  
in polyethylene bags or glass jars at room temperature

Source of variation	Degrees of freedom	Sum of squares	F value
Replication	2	19.18	1.96 NS
Treatments (T)	5	7766.12	317.63 **
Packaging (P)	1	5864.41	1199.27 **
T•P	5	29.65	29.65 NS
Storage time (S)	6	206823.43	34129.23 **
S•T	30	2432.92	80.29 **
S•P	6	2547.35	420.36 **
S•T•P	30	399.29	13.18 **
Contrast			
Linear Storage	1	197076.88	99999.99 **
Quadratic Storage	1	8654.74	8562.85 **

NS Not significant at 5% level

\*\* Significant at 1% level

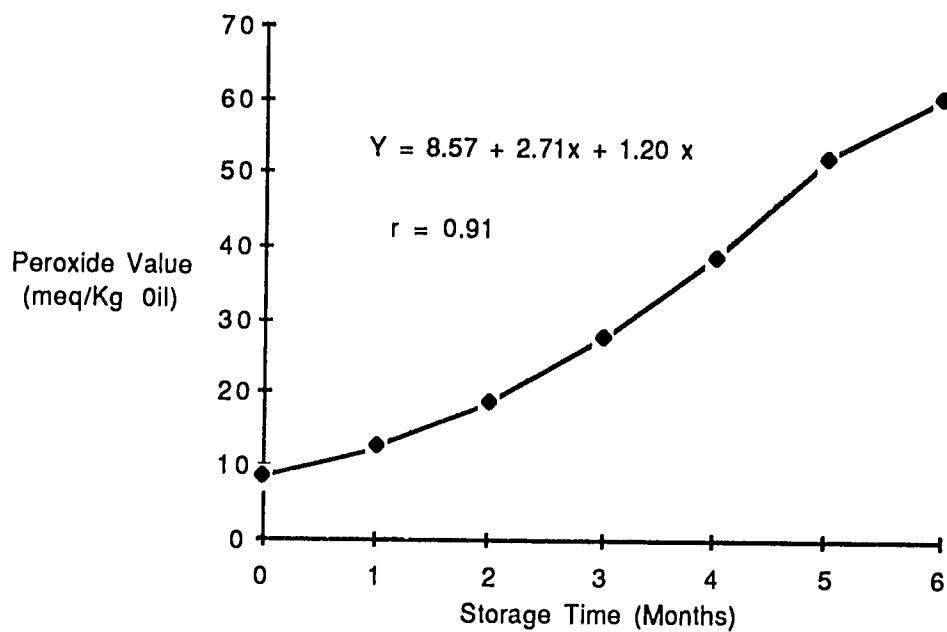


Figure 2. Relationship between storage time and peroxide value of roasted peanuts stored for a period of 0-6 months in polyethylene bags or glass jars at room temperature

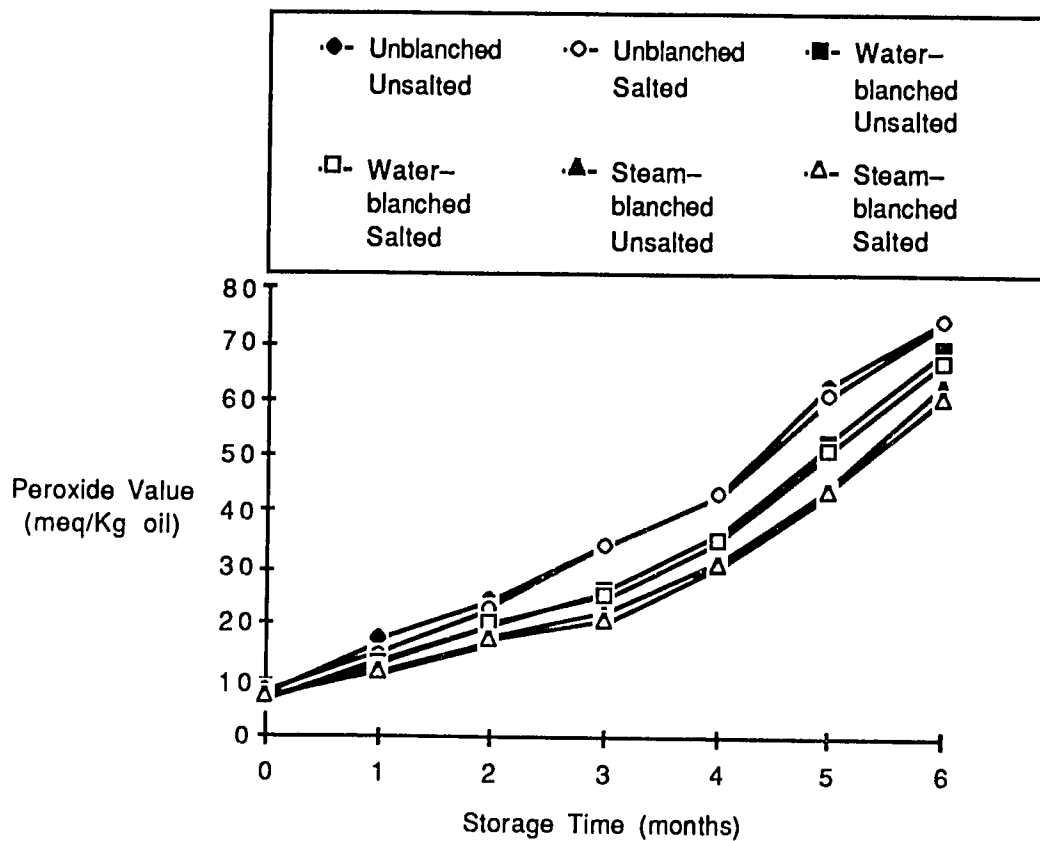


Figure 3. Effects of storage time on peroxide values of roasted peanuts stored for a period of 0-6 months in polyethylene bags or glass jars at room temperature



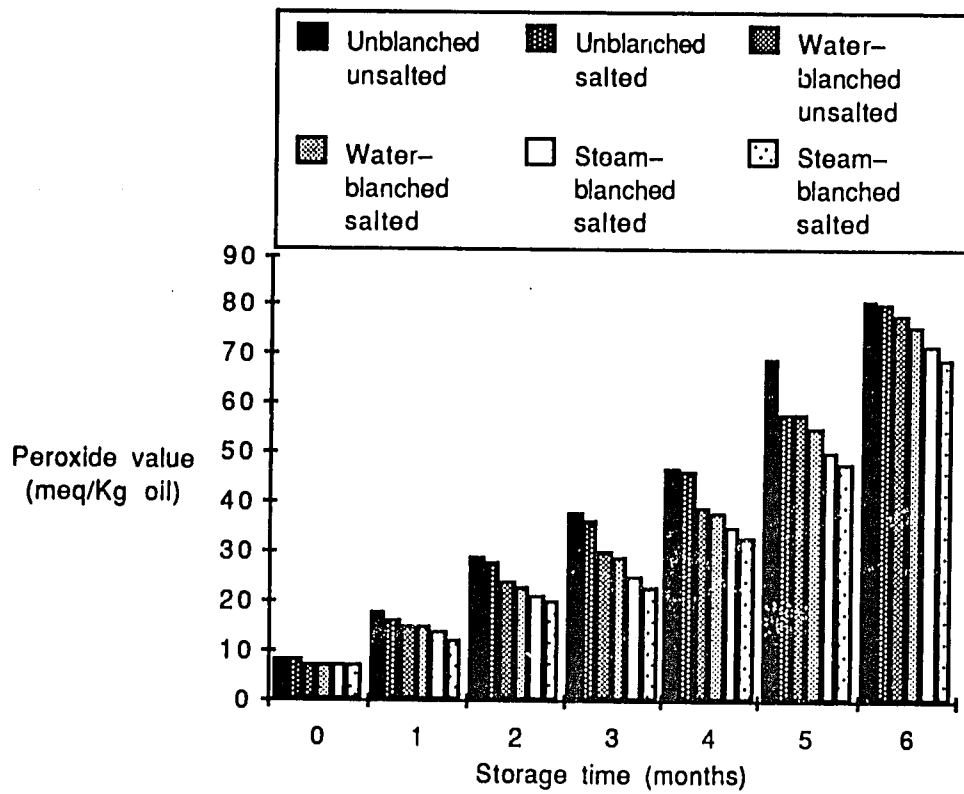


Figure 4. Effects of storage time on peroxide values of roasted peanuts packed in polyethylene bags and stored at room temperature for a period of 0–6 months

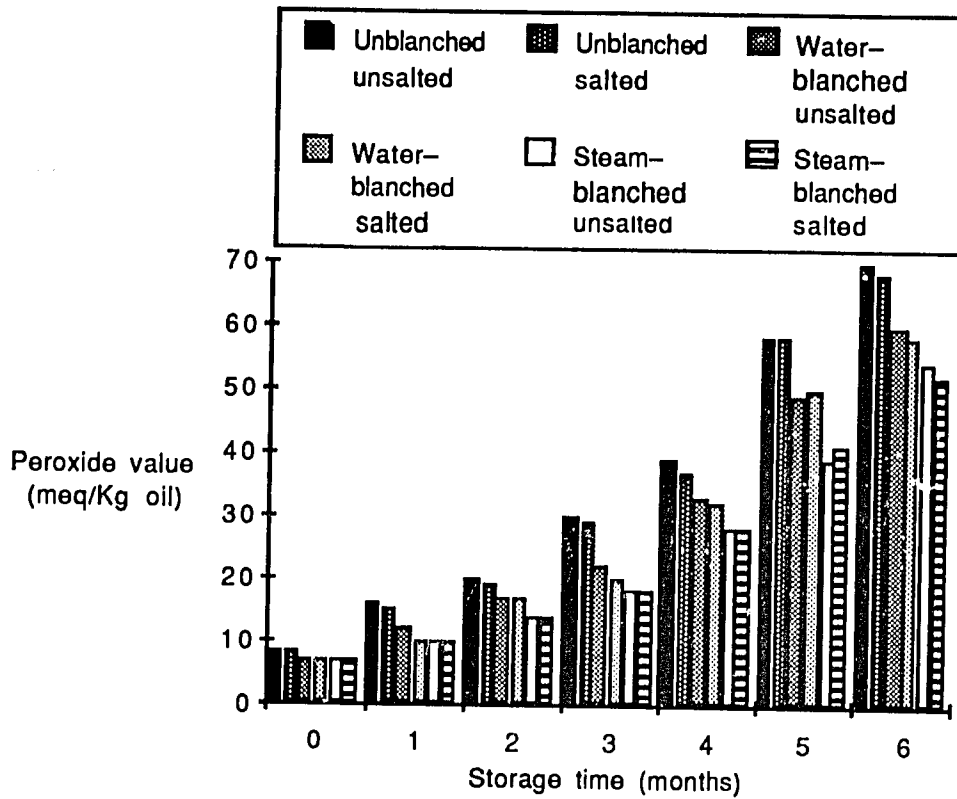


Figure 5. Effects of storage time on peroxide value of roasted peanuts packed in glass jars and stored at room temperature for a period of 0-6 months

Table 3

Combined means of peroxide values and free fatty acid numbers of roasted peanuts<sup>1</sup> stored for a period of 0-6 months in poly ethylene bags or glass jars at room temperature

Treatments	peroxide value meq/Kg oil	free fatty acid (oleic%)
Unblanched unsalted	37.93 a <sup>2</sup>	.14 a
Unblanched salted	36.57 a	.13a
Water-blanched unsalted	32.21 b	.11a
Water-blanched salted	31.35 bc	.11a
Steam-blanched unsalted	28.21 cd	.11a
Steam-blanched salted	27.36 d	.11 a

<sup>1</sup> Values are means of 84 obs. (3 rep., 2 pack., 7 times, and chem. dup.).

<sup>2</sup> Means with the same letter within a column are not significantly different at alpha=0.05 probability.

**Table 4**  
**Effects of storage time on Free Fatty Acid Numbers<sup>1</sup> of roasted peanuts packed in polyethylene bags or glass jars for a period of 0-6 months at room temperature**

Treatment	(Oleic acid %) Storage Time (Month)						
	0	1st	2nd	3rd	4th	5th	6th
Unblanched unsalted	.11	.13	.13	.14	.15	.15	.16
Unblanched salted	.11	.11	.13	.13	.14	.15	.16
Water-blanched unsalted	.11	.11	.11	.11	.11	.11	.11
Water-blanched salted	.11	.11	.11	.11	.11	.11	.11
Steam-blanched unsalted	.11	.11	.11	.11	.11	.11	.11
Steam-blanched salted	.11	.11	.11	.11	.11	.11	.11

<sup>1</sup> Means of three replications, each replication determined in duplicate

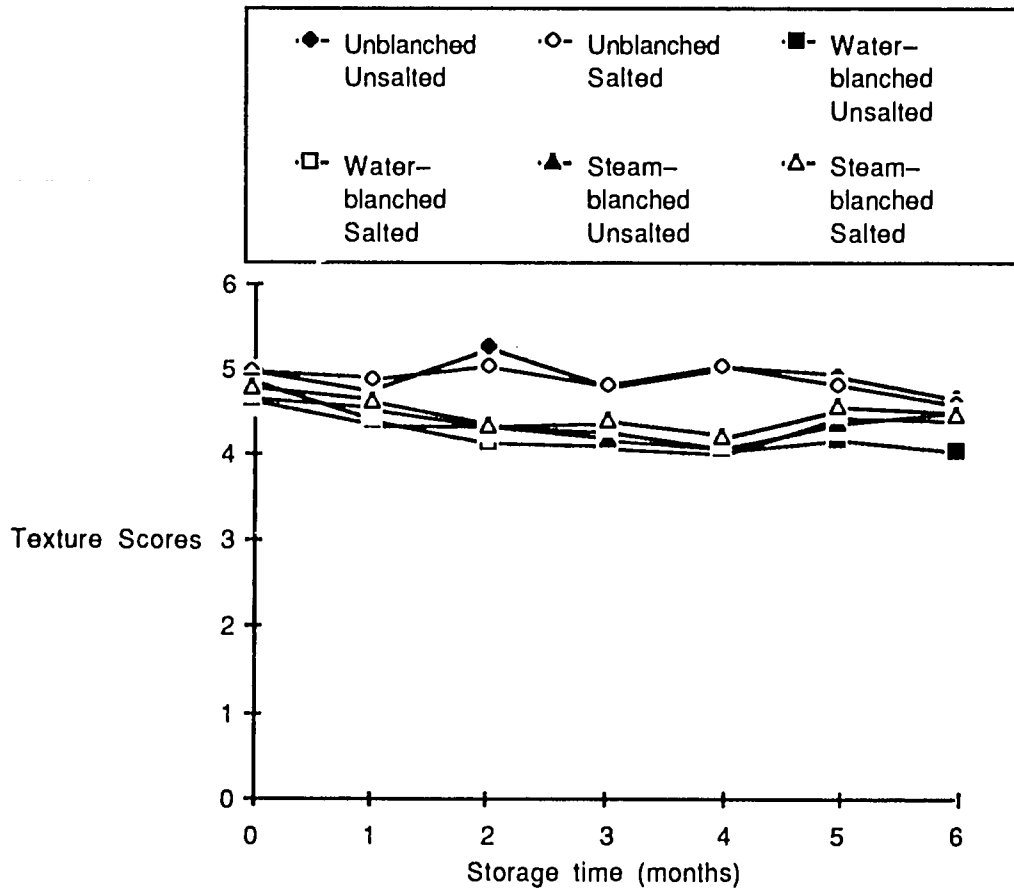


Figure 6. The Influence of storage time on the texture of roasted peanuts, stored for a period of 0-6 months in polyethylene bags or glass jars at room temperature, as detected by the Sensory Evaluation

Table 5

Combined Means of sensory parameters and objective measurements (texture and color) of roasted peanuts stored for a period of 0-6 months in polyethylene bags or glass jars at room temperature

Treatments	Subjective measures <sup>1</sup>			Objective measures <sup>3</sup>	
	Texture	Flavor	Color	Degree of Lightness	Texture Force In Pounds
Unblanched unsalted	4.89 a <sup>2</sup>	3.61 d	5.13 a	53.73 a	561 c
Unblanched salted	4.85 a	3.64 d	5.15 a	51.59 b	575 b
Water-blanched unsalted	4.25 c	3.96 c	4.10 b	50.82 c	602 a
Water-blanched salted	4.34 bc	4.09 bc	4.19 b	50.37 d	597 a
Steam-blanched unsalted	4.38 bc	4.18 b	4.13 b	47.40 e	582 b
Steam-blanched salted	4.48 b	4.45 a	4.16 b	47.10 f	575 b

<sup>1</sup> Values reported are means of 30–55 panelists employing 6-point hedonic scale with 1= dislike very much to 6= like very much.

<sup>2</sup> Means in the same column with the same letter are not significantly different by Duncan's Multiple Range Test with alpha = 0.05 probability

<sup>3</sup> Values are means of 84 observations

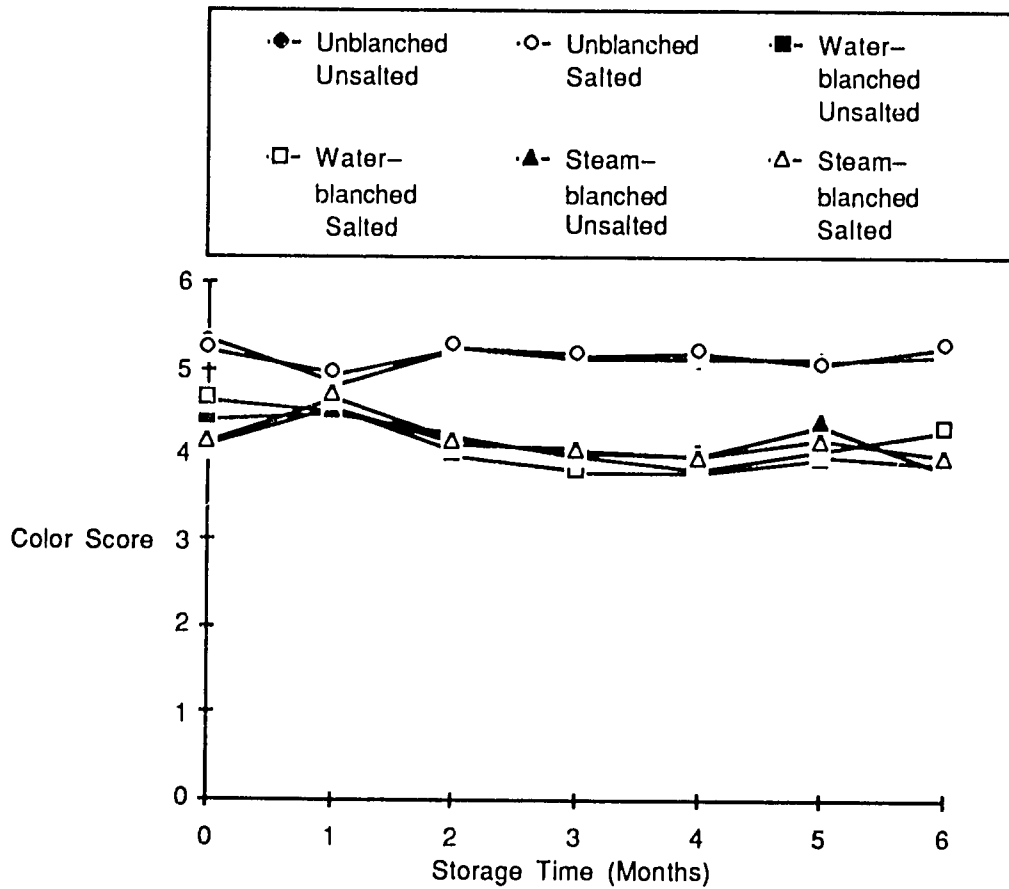


Figure 7. The Influence of storage time on the color of roasted peanuts, stored for a period of 0-6 months in polyethylene bags or glass jars at room temperature, as detected by Sensory Evaluation

The effect of storage time on the texture of the roasted peanuts (Fig. 8 and 9) showed that there were slight changes in the texture with the storage time for all treatments. There were differences between roasted peanuts packed in polyethylene bags and roasted peanuts packed in glass jars. Table 4 shows that there were significant differences between salted and unsalted peanuts for the unblanched peanuts, but there were no significant differences between salted and unsalted peanuts for the blanched peanuts. The unblanched peanuts showed the least values. The water-blanched peanuts had higher values compared with steam-blanched peanuts.

Correlation coefficients among storage time, free fatty acids, peroxide value and objective measurements for color and texture (Table 6) showed that for storage time, there were significant positive correlations with free fatty acid numbers and peroxide values, and negative correlation with texture. For free fatty acids there were positive correlations with peroxide value and color, and negative correlation with texture. For peroxide value there was a negative correlation with color and positive correlation with texture.

#### **Nutritional Improvement of Sorghum-based Kisra Using Defatted Peanut Flour (A. M. Ahmed and B. Singh)**

Kisra is a thin, pancake-like leavened bread made from the sorghum flour. It is the predominant staple in the diet of most people in the sorghum-growing regions of the Sudan. Peanut products (e.g., paste) are commonly used with kisra. The incorporation of defatted peanut flours (after extraction of the oil) may be considered for the purpose of increasing the protein content of sorghum-based flour. A combination of these two common commodities has a potential for acceptance. In Sudan, peanut cake (after extraction with oil) is usually exported. It is possible, though, that the cake could be converted into flour for local consumption. Advantages for developing such technology will be two fold: (1) enhancing the protein level in protein-deficient regions and (2) reducing the amount of food imported. It is understood, however, that these efforts have to be supported by the national government as well as international agencies involved in improving the nutritional status of the Sudanese population. The objectives for these studies include incorporation of defatted peanut flour commercially available in the United States with sorghum flour into kisra. It is assumed that such defatted flour could be feasibly produced in Sudan with minor changes in the existing technology. The result of this study can lead to the further application of available technology to the Sudanese situation.

'Dabar', a commonly grown cultivar in SAT Africa, was obtained from Sudan. 'Dabar' was included as a check for its acclaimed popularity as a good kisra maker. Seeds of six grain sorghum cultivars ('A-TX 623 X CS 3541', and 'A-155 X [120 X 7000]', '77 CS5', 'A-155 X TAN 430', 'A155 X [77 CS1 X TX 430]' and 'A-160 X 77 CS1') were obtained from the Texas Agricultural Experiment Station Sorghum Nursery in 1986. The kisra-making qualities of these seven sorghum cultivars were evaluated. Defatted peanut flour was obtained from Flavored-Nuts Company (North Carolina Division, Seabrook Blanching Corporation) for use as an amendment to the sorghum flour. The starter for fermentation was obtained from Sudan in a powder form (sun-dried) and was typical of that normally used by the Sudanese for making kisra. It was derived from wild yeasts naturally occurring in sorghum flours. Grain samples from all entries were properly cleaned and then ground in a Lee Household flour mill model S-600 (Lee Engineering Co., Milwaukee, WI). Dried starter (120 g) and 480 ml of water were added to 400 g of each sorghum flour. After a thorough mixing, the combinations were incubated in a fermentation cabinet at 27°C until the pH had reached 3.9-4.0 and then freeze-dried for later uses.

Results from preliminary experiments indicated that sorghum cultivars 'A-160 X 77 CS1', 'A-155 X TAN 430' and 'A-TX 623 X CS 3541' gave the best kisra quality. An experiment to evaluate the kisra-making quality by incorporation of the flours of these cultivars with defatted peanut flour was conducted. The basic ingredients used for development of kisra were different ratios: 100%, 90%, 85%, 80%, 75%, and 70% sorghum flour incorporated with 0%, 10%, 15%, 20%, 25%, and 30% defatted peanut flour, respectively.



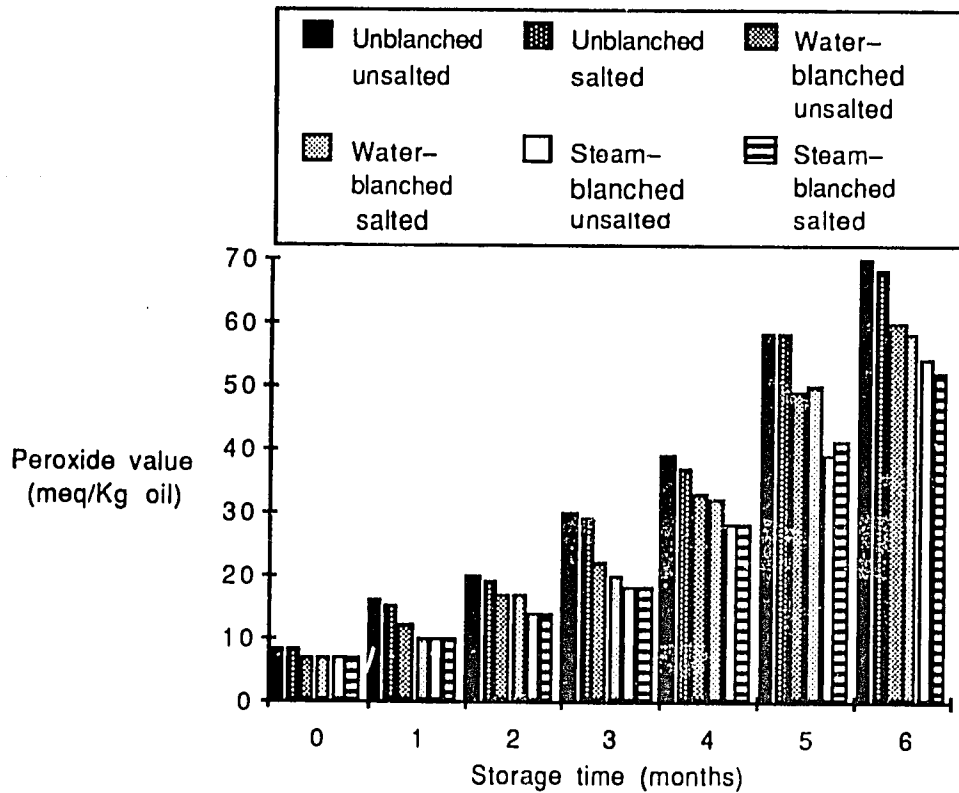


Figure 8. Effects of storage time on peroxide value of roasted peanuts packed in glass jars and stored at room temperature for a period of 0-6 months

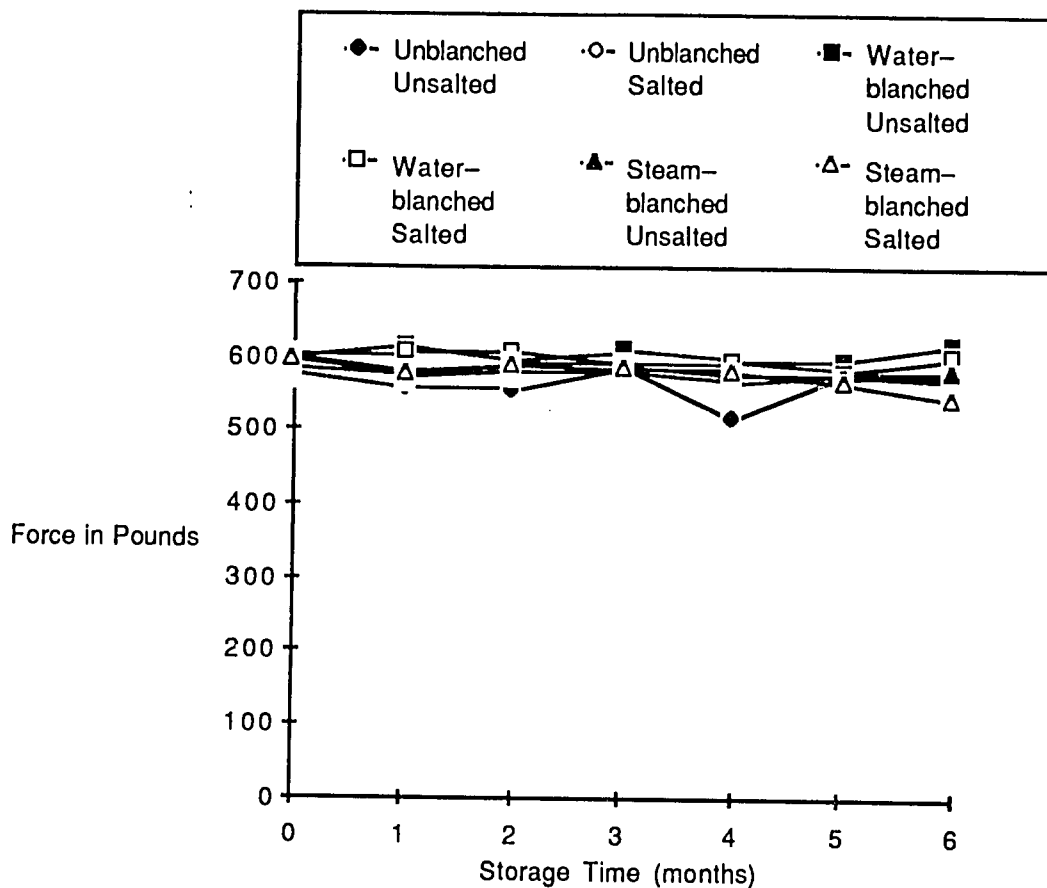


Figure 9. The Influence of storage time on the Texture of roasted peanuts, stored for a period of 0-6 months in polyethylene bags or glass jars at room temperature, as measured by Model TP-4 C Texturepress

Table 6

Correlation coefficients among treatments, packaging materials, storage time, free fatty acids, peroxide value, degree of lightness and objective measures of texture for stored roasted peanuts

Characters	Packaging materials	Storage time	Free Fatty acids	Peroxide value	Degree of lightness	Texture objective
Treatments	0.00	0.00	-0.66**	-0.18**	-0.81**	0.10*
Packaging materials		0.00	0.00	-0.16**	-0.02	-0.05
Storage time			0.35**	0.93**	-0.07	-0.11*
Free fatty acids				0.49**	0.48**	-0.23**
Peroxide value					0.10*	-0.09*
Degree of lightness						-0.06
Texture objective						

\* Significant at 0.05 level of probability

\*\* Significant at 0.01 level of probability

Fermented dough and kiswa were prepared in the traditional way as used by a typical Sudanese housewife. Composite blends of sorghum flour and defatted peanut flour (e.g., 90:10 g per 90% composite blend) were mixed with approximately 120 ml of water in a stainless steel beaker with 30 g of the starter. After a thorough mixing, samples were incubated in a fermentation cabinet at 27°C for 18 hr. At the end of these fermentation processes, pH ranged from 3.7 to 4.0. Just before baking, another 60 ml water were added to the dough in order to bake it on a hot plate at 150-160°C into thin sheets (42 g) within 1.5-2 min. For chemical analysis, a representative sample of these sheets was cut into small pieces and dried in a freeze dryer. Fig. 3 illustrates the overall process for fortified kiswa products.

Data on baking-ease, acceptability, and keeping quality of kiswa were collected. Sorting out preferences or acceptability by color, texture, and taste parameters was done using the same procedures as above. Moisture content of the individual flours and composite flours was determined using an air-oven method at 130°C as described in the AACC standard methods (AACC, 1982). The micro-kjeldahl procedure was used for crude protein. Crude fiber and ash were determined by AACC methods (1982). For amino acid analyses, samples were hydrolysed with 6N HCl and then determinations were made using Dionex Amino acid analyzer.

In vitro digestibility of the samples was determined using pancreatin-pepsin digestion system. Sugars were determined using HPLC system (Perkin-Elmer, model 7500). The Brabender Visco-Amylograph was used to compare the cooking characteristics of the fermented and unfermented flours. The initial concentration of the slurry was 10% (d.w.b.). Pasting temperature, peak viscosity, peak setback and stability of flour paste were recorded.

Additions of different levels of the defatted peanut flour (Table 7) appeared to decrease the residues collected from the surface of the hot plate significantly and, hence, increase the yield of kiswa and thus the baking-ease.

The effects of increasing the levels of the defatted peanut flour on color and texture were encouraging as evaluated by the panelists (Table 7). This might be attributed to the whiter color of the peanut flour. Panelists were not able to distinguish the differences in taste of peanut flour at levels up to 30 % defatted peanut flour additions.

The keeping quality judged by acceptability scaled from like to dislike by a panel test after 24 hr of storing at room temperature showed that there were significant decreases in the scores of the product as the defatted peanut flour fortification increased (Table 7). However, since most of the Sudanese families prepare kiswa for each day, a nutritious food should not be rejected on the basis of keeping quality if it rates well on baking-ease, color, texture and taste.

Amylograph curves for the four cultivars fortified with defatted peanut flour are presented in Fig. 10, 11, 12 and 13. It seemed that the changes in the heights of peak viscosities for cultivar 'Dabar' (Fig. 11) were slightly decreased due to the addition of different levels of defatted peanut flour, while the height of the peak viscosities for sorghum cultivars 'A-160 X 77 CS1', 'A-TX 623 X CS 3541', 'A-155 X TAN 430' were drastically reduced due to fortification with defatted peanut flour.

The ash contents for the four cultivars ('Dabar', 'A-155 X TAN 430', 'A-TX 623 X CS 3541' and 'A-160 X 77 CS1') were significantly different within the cultivars due to fortification (Tables 8, 9, 10 and 11). The highest increase of ash appeared in cultivar 'A-155 X TAN 430' (44.72% at 30% peanut flour level), while the lowest increase was in 'Dabar' (10.95% at the same level). The increases at the same level of fortification in cultivars 'A-TX 623 X CS 3541' and 'A-160 X 77 CS1' were 17.60 and 12.50%, respectively.

There were substantial increases in the protein of the blends of the four cultivars with increased levels of defatted peanut flour fortification. The protein content increased by 40, 51, 71 and 73% in 'A-TX 623 X CS 3541', 'A 155 X TAN 430', 'A-160 X 77 CS1' and 'Dabar', respectively, at the level of 30% fortification. There were no significant differences in the crude fiber of the blends due to fortification with defatted peanut flour.

**Table 7. Baking-Ease and Sensory Evaluation of *Kisra* from Sorghum Flour 'Dabar' Fortified with Defatted Peanut Flour**

Fortification Level (% Defatted Peanut Flour)	Residues on the Hot Plate (g)	Color*	Texture*	Taste*	Overall Acceptability After * 24 hr. At Room Temperature
0	4.10a**	4.22e	3.62b	3.58a	3.20a
10	4.08a	4.26d	3.68b	3.52a	3.18a
15	3.88b	4.28cd	3.69b	3.57a	3.03b
20	3.80b	4.31bc	3.75ab	3.53a	2.78c
25	3.68c	4.33ab	3.76a	3.56a	2.70cd
30	3.60c	4.35a	3.403a	3.55a	2.63c

\* Means of 8 individuals, observations as rated on hedonic scale of 1–5, where 5 = excellent and 1 = very poor.

\*\* Means of any parameter with the same letter are not significantly different ( $P = 0.05$ ) using Duncan Multiple Range.

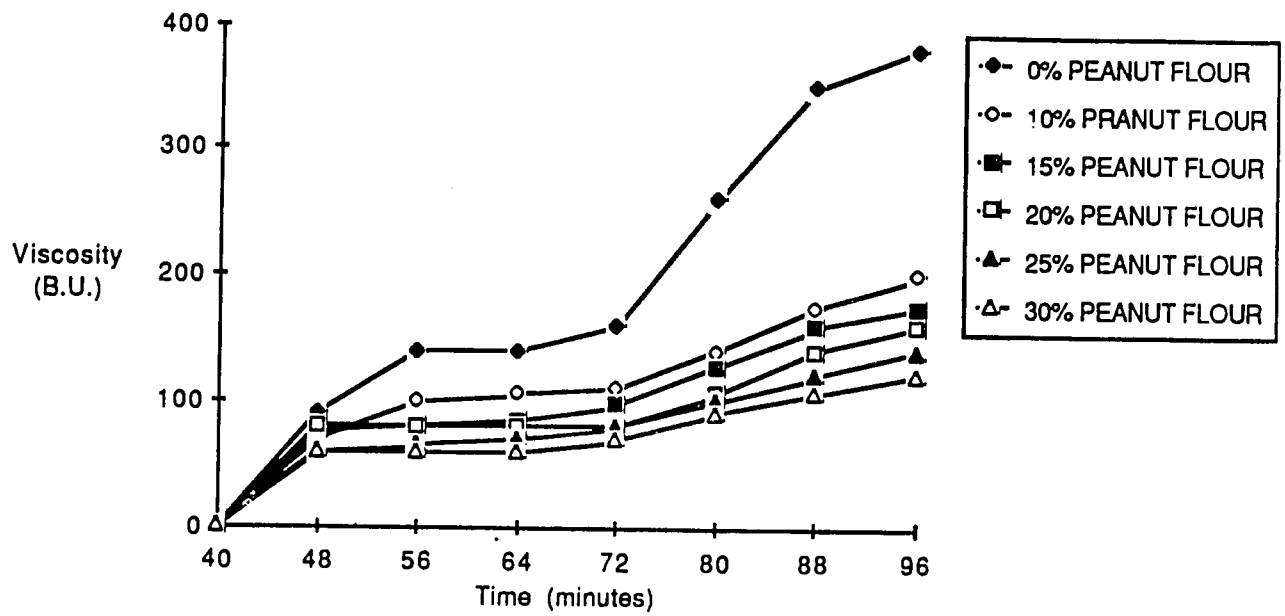


Figure 10. Brabender/Visco/Amylograph Viscosities for Blends of Sorghum Flours from Cultivar 'A-160 X 77 CS1' with Defatted Peanut Flour

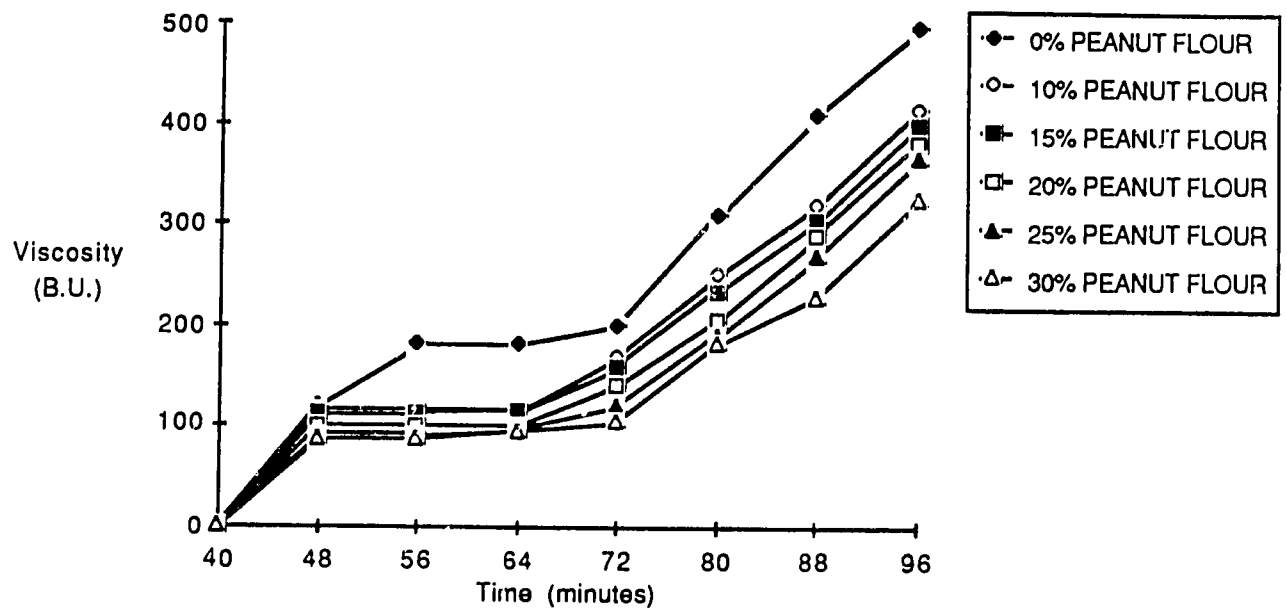


Figure 11. Brabender/Visco/Amylograph Viscosities for Fermented Blends of Sorghum Flours from Cultivar 'Dabar' with Defatted Peanut Flour

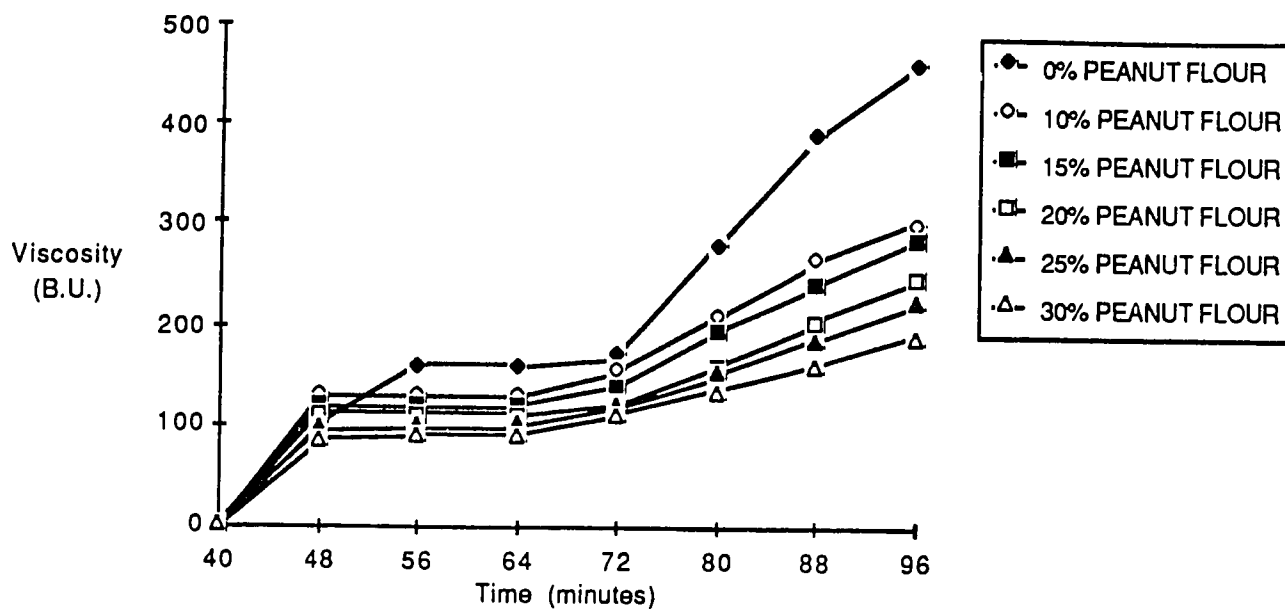


Figure 12. Brabender/Visco/Amylograph Viscosities for Fermented Blends of Sorghum Flours from Cultivar 'A-TX 623 X CS 3541' with Defatted Peanut Flour.



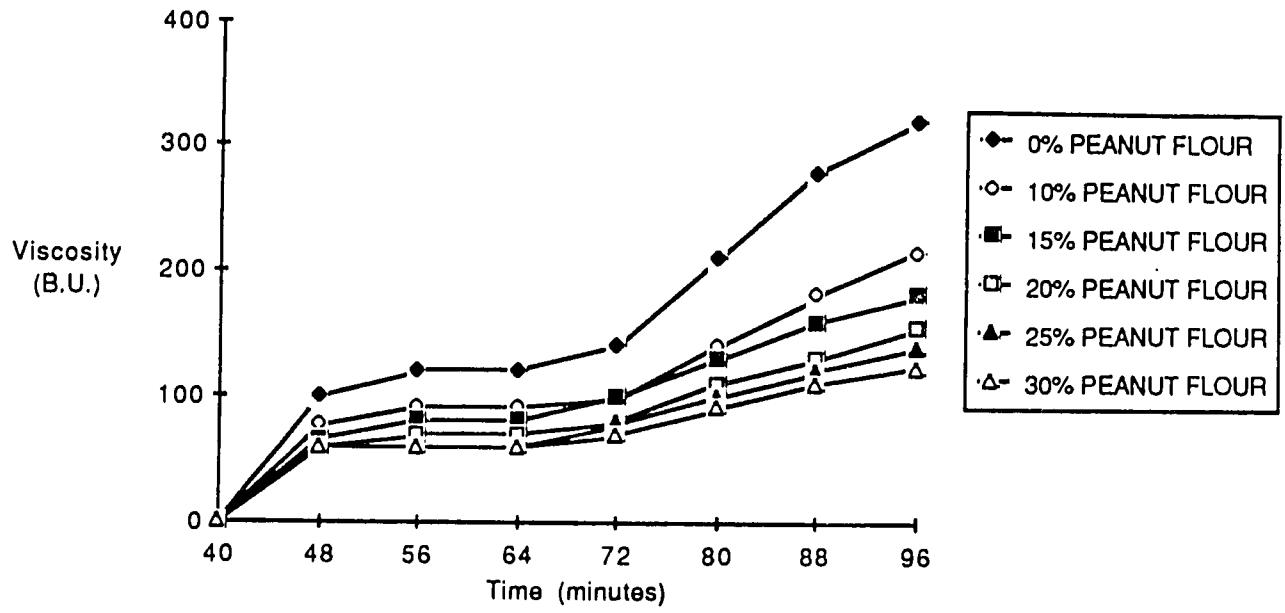


Figure 13. Brabender/Visco/Amylograph Viscosities for Fermented Blends of Sorghum Flours from Cultivar 'A-155 X TAN 430' with Defatted Peanut Flour

**Table 8. Moisture, Ash, Crude Protein and Crude Fiber Content of Flour and Blends with Defatted Peanut Flour of Cultivar 'A-160 x 77 CS1'**

Chemical Composition	% of Defatted Peanut Flour					
	0	10	15	20	25	30
Moisture (%)	4.37e*	10.83a	7.85d	8.46c	9.28b	7.88d
Ash (%)**	1.60c	1.63bc	1.69b	1.78a	1.78a	1.80a
Crude Protein (%)**	12.44f	13.48e	16.71d	17.53c	20.70b	21.30a
Crude Fiber (%)**	3.30c	3.30c	3.31bc	3.32b	3.35a	3.35a

\* Means of any parameter with the same letter are not significantly different (P = 0.05) using Duncan Multiple Range.

\*\* Data were expressed on dry basis.

**Table 9. Moisture, Ash, Crude Protein and Crude Fiber Content of Flour and Blends with Defatted Peanut Flour of Cultivar 'A-TX 623 x CS 3541'**

Chemical Composition	% of Defatted Peanut Flour					
	0	10	15	20	25	30
Moisture (%)	3.11f*	8.18cd	9.15b	7.44e	9.64a	7.88ce
Ash (%)**	1.42d	1.43d	1.54c	1.65b	1.65b	1.67a
Crude Protein (%)**	14.71f	15.08e	15.79d	16.60c	18.42b	20.53a
Crude Fiber (%)**	3.10d	3.15c	3.20b	3.22a	3.22b	3.25a

\* Means of any parameter with the same letter are not significantly different ( $P = 0.05$ ) using Duncan Multiple Range

\*\*Data were expressed on dry basis.

Table 10. Moisture, Ash, Crude Protein and Crude Fiber Content of Flour and Blends with Defatted Peanut Flour of Cultivar 'A-155 x TAN 430'

Chemical Composition	% of Defatted Peanut Flour					
	0	10	15	20	25	30
Moisture (%)	2.41d*	9.25ab	9.46a	9.25ab	9.00bc	8.87c
Ash (%)**	1.61e	2.07d	2.09c	2.25b	2.25b	2.33a
Crude Protein (%)**	14.38f	14.93e	15.54d	17.87c	19.55b	21.66a
Crude Fiber (%)**	3.10b	3.20ab	3.25a	3.25a	3.30a	3.30a

\* Means of any parameter with the same letter are not significantly different ( $P = 0.05$ ) using Duncan Multiple Range

\*\* Data were expressed on dry basis.

Table 11. **Molsture, Ash, Crude Protein and Crude Fiber Content of Flour and Blends with Defatted Peanut Flour of Cultivar 'Dabar'**

Chemical Composition	% of Defatted Peanut Flour					
	0	10	15	20	25	30
Moisture (%)	2.60f*	7.37e	7.85c	8.36b	9.15a	7.50de
Ash (%)**	1.37c	1.45b	1.45b	1.48ab	1.51a	1.52a
Crude Protein (%)**	12.20f	13.28e	15.91d	17.33c	19.85b	21.10a
Crude Fiber (%)**	3.60c	3.60c	3.63b	3.65ab	3.67a	3.67a

\* Means of any parameter with the same letter are not significantly different ( $P = 0.05$ ) using Duncan Multiple Range

\*\* Data were expressed on dry basis.

The results in Tables 12, 13, 14 and 15 show that there were significant decreases in sucrose content due to fermentation. The sucrose decreased by 27, 39, 50 and 62% for 'A-160 X 77 CS1', 'A-155 X TAN 430', 'A-TX 623 X CS 3541' and 'Dabar', respectively. The decreases in glucose and fructose were not as drastic as those of sucrose. This may be due to hydrolysis of sucrose to its components (glucose and fructose) during the fermentation processes. Also, there were decreases in the amounts of melibiose and maltose during fermentation for all cultivars. Fortification increased the amount of the sucrose by an average of 12.8% at 30% level. There were slight increases in the raffinose content as well as the amount of glucose and fructose as a result of adding more defatted peanut flour. Maltose was not detected and melibiose decreased for unexplained reasons as defatted peanut flour increased. The baking process affected the percentages for all sugars of the four cultivars studied. Sucrose, glucose and fructose decreased but raffinose increased for all cultivars at different levels of blending, while melibiose increased and decreased depending on the cultivar.

The amino acid composition present in the fermented blends and kiswa products with the respective sorghum cultivars are given in Tables 16, 17, 18 and 19. Fermentation of sorghum slightly increased the amount of thirteen amino acids (lysine, serine, proline, threonine, glutamic acid, phenylalanine, methionine, isoleucine, arginine, histidine, leucine, valine and tyrosine). A decrease was recorded in aspartic acid and alanine. Baking the fermented sorghum meal (kiswa) resulted in sharp decreases in methionine, aspartic acid and phenylalanine, and slight decreases in lysine, histidine and glycine. Slight increases were observed in glutamic acid, arginine, valine, glycine and isoleucine. There were negligible decreases in threonine. In the case of composite blends, an increase was observed in all amino acids determined. However, the values of the amino acids decreased upon baking. In all samples, lysine remained the most limiting amino acid. If fermentation is left to proceed naturally, then the fermentation conditions such as temperature, time and natural microflora should determine the nutritional characteristics of the end product. In this study, the increases in lysine and methionine on the fermentation of sorghum meal were negligible compared to a noticeable increase in the fermented blends (Table 16, 17, 18 and 19). Table 20 showed that there is a positive correlation between the increase of protein and the two limiting amino acids (lysine and methionine).

Fermentation of unfortified doughs resulted in some increases and decreases in both leucine/isoleucine and leucine/lysine ratios in sorghum meals depending on cultivar (Tables 21, 22, 23, and 24). Excesses of leucine in the diet inhibit the conversion of tryptophan to nicotinic acid and hence causes pellagra in populations subsisting principally on sorghum. It has been suggested that a leucine/isoleucine ratio higher than 3.0 should be regarded as potentially deleterious. A ratio of 3.0 or less was found in 'A-TX 623 X CS 3451' and 'Dabar' blends containing 30% defatted peanut flour (Tables 22 and 24). Baking of these fermented blends further reduced the ratio, and hence improved the quality of the products. For 'A-155 X TAN 430' and 'A-160 X 77 CS1' the ratios of leucine/isoleucine were higher than the upper level recommended at 30% fortification (Tables 21 and 23). A value of 4.6 was recommended as a safe upper limit for the leucine/lysine ratio. The ratio was satisfactorily achieved by 10% or more fortification level for cultivar 'A-TX 623 X CS 3451', 15% or more for cultivars 'Dabar' and 'A-160 X CS1' and 20% or more for cultivar 'A-155 X TAN 430' (Table 21, 22, 23 and 24). The essential amino acids to total amino acids ( $E/T_N$  ratio) of 2.25 was given as a quality index in the FAO Provisional Pattern (FAO/WHO Ad hoc Expert Committee, 1973). Sorghum flour by itself seemed to manifest sufficient  $E/T_N$  ratio due to its high amounts of leucine and phenylalanine. In Tables 22 and 24, kiswa formulated of 30% fortified fermented blends of 'A-TX 623 X CS 3541' and of 25 and 30% fortified fermented blends of 'Dabar' cultivars showed values below the recommended  $E/T_N$  ratio (2.20 and 2.18, respectively).

In vitro digestibilities of protein of the sorghum flours, fermented sorghum flours, fermented blends and kiswa products are presented in Tables 21, 22, 23 and 24. Fermentation appeared to increase the pepsin-pancreatin digest for all samples. On the other hand, baking fermented doughs into kiswa did not improve the digestibility of these products. Nevertheless, the addition of defatted peanut flour brought considerable improvements to the in vitro digestibilities.

Table 12. Sugar Content of Unfermented, Fermented and Fortified Doughs and *Kisra* from Cultivar 'A-155 x TAN 430'

Sample	Concentration (mg/g) <sup>***</sup>					
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Melibiose
Sorghum Flour	6.44*	4.23	14.39	2.45	–**	1.98
Fermented Dough	4.53	3.41	8.81	1.06	–	1.35
10% Fortified Dough	4.62	4.69	9.99	–	–	0.91
15% Fortified Dough	5.23	4.39	9.15	–	–	0.88
20% Fortified Dough	5.66	4.34	9.42	–	–	–
25% Fortified Dough	5.00	3.41	9.55	–	2.71	–
30% Fortified Dough	4.33	3.77	9.48	–	2.45	–
10% Fortified <i>Kisra</i>	3.40	3.81	5.54	–	0.44	0.39
15% Fortified <i>Kisra</i>	3.73	3.68	5.63	–	0.60	0.44
20% Fortified <i>Kisra</i>	4.23	3.01	5.38	–	0.72	0.98
25% Fortified <i>Kisra</i>	5.10	3.88	6.24	–	0.87	–
30% Fortified <i>Kisra</i>	6.45	3.69	6.84	–	1.38	–

\* Mean of three replications

\*\* No detectable amount

\*\*\* Data expressed on dry basis.

Table 13. Sugar Content of Unfermented, Fermented and Fortified Doughs and *Kisra* from Cultivar 'A-TX 623 x CS 3541'

Sample	Concentration (mg/g) <sup>***</sup>					
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Melibiose
Sorghum Flour	6.76*	4.86	12.41	2.15	-**	2.56
Fermented Dough	5.57	4.53	6.24	1.23	-	1.74
10% Fortified Dough	6.07	5.17	8.14	-	4.55	1.83
15% Fortified Dough	4.45	5.36	9.39	-	3.14	-
20% Fortified Dough	3.80	4.87	10.32	-	2.36	-
25% Fortified Dough	5.48	3.63	6.76	-	1.20	-
30% Fortified Dough	6.10	3.40	8.13	-	2.82	-
10% Fortified <i>Kisra</i>	5.16	3.81	6.15	-	-	1.06
15% Fortified <i>Kisra</i>	4.87	3.37	6.35	-	1.95	0.63
20% Fortified <i>Kisra</i>	3.40	3.24	5.92	-	1.83	-
25% Fortified <i>Kisra</i>	4.15	3.26	5.74	-	1.79	-
30% Fortified <i>Kisra</i>	4.44	3.16	5.54	-	1.04	-

\* Mean of three replications.

\*\* No detectable amount.

\*\*\* Data expressed on dry basis.



Table 14. Sugar Content of Unfermented, Fermented and Fortified Doughs and *Kisra* from Cultivar 'A-160 x 77 CS1'

Sample	Concentration (mg/g) <sup>***</sup>					
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Melibiose
Sorghum Flour	8.50*	4.08	12.70	2.00	-**	2.34
Fermented Dough	6.64	3.23	9.25	1.91	-	2.15
10% Fortified Dough	6.74	4.15	8.93	-	1.93	1.38
15% Fortified Dough	6.81	4.36	7.88	-	2.08	1.26
20% Fortified Dough	6.85	3.95	8.19	-	2.71	1.11
25% Fortified Dough	6.89	3.79	8.54	-	3.55	1.16
30% Fortified Dough	6.82	3.52	8.97	-	4.37	1.02
10% Fortified <i>Kisra</i>	5.65	4.09	8.40	-	-	0.98
15% Fortified <i>Kisra</i>	5.72	4.21	8.48	-	2.57	1.37
20% Fortified <i>Kisra</i>	5.26	3.50	8.57	-	3.11	1.86
25% Fortified <i>Kisra</i>	5.42	3.41	8.34	-	3.62	1.74
30% Fortified <i>Kisra</i>	5.54	3.24	8.10	-	4.22	1.03

\* Mean of three replications

\*\* No detectable amount

\*\*\* Data expressed on dry basis.

Table 15. Sugar Content of Unfermented, Fermented and Fortified Doughs and *Kisra* from Cultivar 'Dabar'

Sample	Concentration (mg/g) <sup>***</sup>					
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Melibiose
Sorghum Flour	7.13*	5.26	12.89	2.20	-**	2.29
Fermented Dough	5.16	4.16	5.73	1.40	-	1.75
10% Fortified Dough	5.74	4.68	6.45	-	2.83	1.37
15% Fortified Dough	5.98	4.91	6.76	-	2.74	1.38
20% Fortified Dough	6.25	5.04	7.14	-	3.30	-
15% Fortified Dough	6.37	5.16	7.48	-	2.49	-
30% Fortified Dough	6.43	5.20	7.67	-	3.21	-
10% Fortified <i>Kisra</i>	4.66	4.27	6.57	-	0.82	-
15% Fortified <i>Kisr</i>	4.93	4.38	6.65	-	1.71	1.12
20% Fortified <i>Kisra</i>	5.11	4.45	6.80	-	1.89	1.25
25% Fortified <i>Kisra</i>	5.42	4.57	6.98	-	2.09	-
30% Fortified <i>Kisra</i>	6.27	4.65	7.08	-	2.21	-

\* Mean of three replications

\*\* No detectable amount

\*\*\* Data expressed on dry basis.

Table 16. Amino Acid Compositions of Unfermented, Fermented Flours, Fermented Blends and Klsra from Sorghum Cultivar 'Dabar' (mg/g sample)\*\*

Amino acids	Sorg. flour	Ferm dough	Baked klsra	10% D. P. F*		15% D. P. F		20% D. P. F		25% D. P. F		30% D. P. F.	
				Ferm	Klsra	Ferm	Klsra	Ferm	Klsra	Ferm	Klsra	Ferm	Klsra
ASP	7.96	8.25	8.12	11.36	10.87	11.82	11.23	12.12	11.61	13.22	12.61	15.43	14.79
THR	2.94	3.50	3.16	3.52	3.22	4.81	4.71	5.95	5.78	6.12	5.99	7.59	7.36
SER	5.65	5.91	5.65	6.10	5.48	7.21	6.43	8.21	7.52	8.79	6.43	9.16	8.71
GLU	23.17	24.57	23.52	27.41	26.71	28.94	27.46	31.43	29.40	33.33	32.11	35.12	33.42
PRO	9.49	9.12	9.01	10.91	10.00	11.41	10.76	12.10	11.61	12.23	11.82	10.61	10.45
GLY	3.84	4.57	4.21	4.92	4.39	5.11	4.89	5.10	4.88	5.12	4.94	5.42	5.62
ALA	10.65	10.50	10.26	10.49	9.74	11.26	10.44	11.86	10.71	11.71	11.29	11.42	11.02
MET	1.15	1.43	1.28	1.56	1.04	1.74	1.75	2.40	2.40	2.43	2.20	2.76	2.22
ILE	2.71	2.80	2.71	3.10	2.88	3.81	3.53	4.73	5.08	4.81	5.21	6.21	6.67
LEU	13.28	13.37	13.59	14.73	14.67	15.10	15.11	15.40	15.93	16.14	16.24	17.02	17.94
TYR	3.36	3.43	3.62	3.51	3.42	4.10	3.98	4.40	4.12	4.39	4.12	4.67	4.15
HIS	2.37	2.55	2.32	2.72	2.42	3.47	3.71	4.63	3.85	5.17	4.71	5.21	4.80
LYS	2.17	2.24	2.22	2.39	2.24	3.67	3.90	5.43	4.90	6.65	5.73	7.82	6.59
VAL	4.87	5.34	5.95	5.68	6.78	5.42	5.56	5.78	5.95	6.42	6.94	7.82	4.05
PHE	3.94	5.79	5.41	6.15	5.61	7.14	6.30	7.51	6.47	7.81	6.64	7.92	6.82
ARG	2.73	3.54	3.52	3.82	3.90	3.99	3.97	4.24	4.11	4.62	4.56	4.80	4.68

\*D. P. F. = Defatted peanut flour. ; Ferm. = Fermented.

\*\* Data expressed on dry basis. Each value is a mean of two replications.

**Table 17. Amino Acid Compositions of Unfermented, Fermented Flours, Fermented Blends and Kisra from Sorghum Cultivar 'A-155 x TAN 430' (mg/g sample)\*\***

AMINO ACIDS	SORG. FLOUR	FERM. DOUGH	BAKED 10% D. P. F.		15% D. P. F.		20% D. P. F.		25% D. P. F.		30% D. P. F.		
			KISRA FERM.	KISRA	FERM. KISRA	KISRA	FERM. KISRA	KISRA	FERM. KISRA	KISRA			
ASP	7.24	7.82	7.36	10.41	10.07	11.85	12.31	15.66	14.33	15.42	14.68	17.52	17.63
THR	2.50	2.79	2.61	3.14	3.02	3.17	3.54	4.10	3.89	4.05	3.63	4.07	3.85
SER	5.23	5.64	5.50	6.54	6.76	7.06	7.27	8.56	7.97	8.22	8.29	9.31	9.38
GLU	22.07	23.55	23.19	26.84	26.64	28.49	30.93	35.32	33.30	33.15	33.03	36.59	37.26
PRO	8.50	9.05	8.88	9.28	9.10	9.36	9.62	11.02	9.99	10.14	9.75	10.71	10.35
GLY	2.96	3.42	3.19	4.72	4.93	5.65	5.98	7.23	7.05	7.43	7.11	8.28	8.66
ALA	9.64	10.57	10.38	10.34	9.28	10.34	9.97	11.89	10.12	10.84	9.34	11.33	10.43
MET	1.87	1.67	1.60	2.35	1.83	3.67	2.50	4.52	3.05	5.21	4.60	6.51	6.17
ILE	2.18	2.56	2.33	3.17	2.27	3.68	3.20	4.01	3.60	3.89	3.45	4.19	3.34
LEU	13.00	13.98	13.28	14.70	13.11	15.32	14.28	15.78	14.68	15.60	13.92	16.65	15.04
TYR	3.09	3.16	2.86	4.07	3.63	4.46	4.21	3.84	5.00	5.14	4.72	5.71	5.46
HIS	2.18	2.36	2.08	2.84	2.71	3.21	3.03	3.66	3.39	3.51	3.33	3.98	3.65
LYS	2.02	2.16	2.13	2.71	2.42	3.38	2.97	4.04	3.64	4.21	3.82	4.36	3.95
VAL	4.50	5.03	5.24	5.49	5.87	5.58	6.51	6.73	6.74	7.54	7.67	8.42	8.55
PHE	3.50	5.18	5.48	5.83	6.26	7.34	6.48	9.07	6.91	6.91	6.48	12.53	7.77
ARG	1.67	2.67	2.69	5.16	5.88	11.73	8.54	8.11	10.55	8.47	8.44	10.05	9.65

\*D. P. F. = Defatted Peanut Flour. ; Ferm. = Fermented.

\*\* Data expressed on dry basis. Each value is a mean of two replications.

**Table 18. Amino Acid Compositions of Unfermented, Fermented Flours, Fermented Blends and Kisra from Sorghum Cultivar 'A-TX 623 x CS 3541' (mg/g sample)\*\***

Amino acids	SORG.	FERM.	BAKED	10% D. P. F.*		15% D. P. F.		20% D. P. F.		25% D. P. F.		30% D. P. F.	
	FLOUR	DOUGH	KISRA	FERM	KISRA	FERM.	KISRA	FERM.	KISRA	FERM.	KISRA	FERM.	KISRA
ASP	6.51	6.13	5.79	10.35	9.65	11.44	9.21	12.41	9.41	15.23	11.92	16.23	11.84
THR	2.21	2.39	2.29	2.65	2.58	2.91	2.69	2.81	2.42	3.43	3.08	3.71	3.07
SER	4.25	4.38	4.52	5.37	5.68	6.27	5.85	6.19	5.86	7.77	6.96	8.55	7.19
GLU	17.64	18.11	17.89	21.13	21.92	24.21	25.75	26.35	24.95	29.46	29.06	32.44	29.83
PRO	6.54	6.98	6.97	6.75	7.28	7.61	8.16	9.59	8.44	8.33	8.56	8.05	8.68
GLY	2.78	2.96	2.68	4.60	4.84	5.57	4.96	4.90	4.52	7.45	6.19	8.33	6.39
ALA	7.64	8.17	7.79	7.73	7.81	8.27	6.76	9.89	9.04	8.69	7.38	9.08	6.91
MET	1.54	1.82	1.56	2.15	1.88	2.48	2.23	2.76	1.91	3.31	2.92	4.02	3.73
ILE	2.27	2.24	2.21	2.58	2.20	2.77	2.49	3.57	3.09	3.40	2.94	4.52	3.85
LEU	10.98	11.02	10.82	10.95	10.52	11.65	10.05	14.21	11.92	12.62	11.03	13.26	10.40
TYR	2.41	2.69	2.71	3.33	3.34	3.84	3.22	5.47	3.51	4.41	3.96	4.98	4.05
HIS	2.11	2.03	2.03	2.41	2.40	2.71	2.45	3.36	2.33	3.33	2.84	3.59	2.85
LYS	1.91	1.94	1.82	2.73	2.52	3.03	2.81	3.52	3.31	4.06	3.53	4.52	3.76
VAL	3.97	4.59	4.97	4.79	5.22	5.69	5.49	6.08	6.55	7.07	7.31	6.44	7.44
PHE	3.97	4.59	4.97	4.78	5.22	5.46	5.68	5.78	6.08	7.28	7.31	7.45	7.45
ARG	1.95	2.35	2.83	4.79	6.10	6.43	4.79	9.06	5.32	9.65	7.64	10.65	6.63

\*D. P. F. = Defatted peanut flour. ; Ferm. = Fermented.

\*\* Data expressed on dry basis. Each value is a mean of two replications.

**Table 19. Amino Acid Compositions of Unfermented, Fermented Flours, Fermented Blends and Kisra from Sorghum Cultivar 'A-160 x 77 CS1' (mg/g sample)\*\***

Amino acids	SORG.	FERM BAKED		10% D. P. F*		15% D. P. F		20% D. P. F		25% D. P. F		30% D. P. F	
	FLOUR	DOUG†	KISRA	FERM.	KISRA	FERM.	KISRA	FERM.	KISRA	FERM.	KISRA	FERM.	KISRA
ASP	6.76	6.55	6.21	10.37	9.15	12.96	11.50	14.16	12.55	16.33	15.12	17.89	15.75
THR	2.35	2.43	2.36	2.68	2.77	3.34	2.90	3.48	3.36	3.96	3.48	4.02	3.68
SER	4.87	5.04	4.92	6.17	5.99	8.03	6.89	7.64	7.63	8.67	8.09	9.08	8.55
GLU	19.93	20.68	20.24	25.81	27.41	28.95	27.73	30.46	33.43	34.01	33.84	36.49	34.59
PRO	7.53	7.81	7.13	8.76	8.45	9.44	8.85	9.48	10.37	9.86	9.95	10.37	10.05
GLY	2.76	2.81	3.04	4.63	5.06	5.90	5.41	6.42	6.24	7.64	7.23	8.43	7.56
ALA	8.78	9.30	6.22	9.94	6.16	10.66	9.04	10.17	9.01	10.69	9.77	10.95	9.82
MET	1.26	1.80	1.65	2.06	1.37	2.85	2.06	3.43	2.22	4.74	2.29	5.43	5.21
ILE	2.05	2.17	2.12	2.35	2.70	3.10	2.61	3.45	3.05	3.96	3.16	4.13	3.64
LEU	11.75	12.41	11.92	13.51	12.63	14.77	12.75	14.58	13.28	15.86	14.27	16.26	14.67
TYR	2.83	2.97	2.62	3.80	3.43	4.58	3.97	4.67	4.41	5.32	4.69	5.79	4.90
HIS	2.00	2.11	2.11	2.53	2.52	3.15	2.81	3.20	3.07	3.65	3.35	3.86	3.50
LYS	2.02	2.45	2.41	3.01	2.71	3.27	2.80	3.48	3.02	4.11	3.78	4.57	3.87
VAL	3.20	3.59	3.41	3.69	3.69	3.89	3.62	4.57	4.31	4.57	4.44	5.21	5.97
PHE	4.32	4.28	4.13	3.49	3.30	4.84	4.66	6.41	6.57	4.79	6.61	7.77	5.44
ARG	2.58	3.01	3.11	5.23	4.53	8.04	5.34	7.37	7.24	10.02	8.11	10.85	9.04

\*D. P. F = Defatted peanut flour. ; Ferm. = Fermented.

\*\* Data expressed on dry basis. Each value is a mean of two replications.

Table 20. Relationships Between the Increase in the Protein and the Two Limiting Amino Acids (Lysine and Methionine) at 30% Level of Defatted Peanut Flour

Cultivar	Protein Increase(%)	Lysine Increase (%)	Methionine Increase (%)
'Dabar'	73.00	102.3	11.40
'A-160 x 77 CS1'	71.22	86.5	75.40
'A-TX 623 x CS 3541'	39.60	82.0	9.53
'A-155 x TAN 430'	50.60	34.7	67.60

**Table 21. Ratios of Amino Acids as Quality Indices and In Vitro Digestibility of Sorghum Flour from Cultivar 'A-155 x TAN 430' with Indicated Levels of Defatted Peanut Flour**

Sample	Leucine/Isoleucine	Leucine/Lysine	E/T <sub>N</sub> *	Pepsin-Pancreatin Digest (%)
Sorghum Flour	5.96	6.42	2.69	65.1
Fermented Dough	5.47	6.63	2.65	66.3
Kisra	5.01	6.13	2.68	66.1
10% Blend:				
Fermented Dough	4.63	5.43	2.62	67.3
Kisra	4.77	5.43	2.54	66.3
15% Blend:				
Fermented Dough	4.16	4.54	2.56	68.6
Kisra	4.46	4.80	2.47	67.7
20% Blend:				
Fermented Dough	3.94	3.90	2.53	68.9
Kisra	4.08	4.03	2.45	68.0
25% Blend:				
Fermented Dough	4.02	3.70	2.43	70.1
Kisra	4.03	3.65	2.35	69.7
30% Blend:				
Fermented Dough	3.97	3.82	2.39	71.2
Kisra	4.50	3.81	2.28	70.2
FAO Reference			2.25	

\* Ratio of essential amino acid (E) to total amino acids (T<sub>N</sub>)



**Table 22. Ratios of Amino Acids as Quality Indices and In Vitro Digestibility of Sorghum Flour from Cultivar 'A-TX 623 x CS 3541' with Indicated Levels of Defatted Peanut Flour Fortification**

Sample	Leucine/Isoleucine	Leucine/Lysine	E/T* N	Pepsin-Pancreatin digest (%)
Sorghum Flour	4.83	5.76	2.72	64.3
Fermented Dough	4.92	5.68	2.65	65.2
Kisra	4.89	5.95	2.71	65.1
10% Blend:				
Fermented Dough	4.24	4.01	2.57	66.4
Kisra	4.78	4.18	2.48	64.6
15% Blend:				
Fermented Dough	4.20	3.84	2.53	67.25
Kisra	4.02	3.58	2.41	66.78
20% Blend:				
Fermented Dough	4.00	4.04	2.49	68.2
Kisra	3.86	3.60	2.37	67.9
25% Blend:				
Fermented Dough	3.71	3.11	2.45	69.1
Kisra	3.75	3.13	2.30	68.8
30% Blend:				
Fermented Dough	2.94	3.94	2.38	72.5
Kisra	2.70	2.74	2.20	71.5
FAO Reference			2.25	

\* Ratio of essential amino acids (E) to total amino acids (T )  
N

**Table 23. Ratios of Amino Acids as Quality Indexes and In Vitro Digestibility of Sorghum Flour from Cultivar 'A-160 x 77 CS1' with Indicated Levels of Defatted Peanut Flour**

Sample	Leucine/Isoleucine	Leucine/Lysine	E/T* N	Pepsin-Pancreatin Digest (%)
Sorghum Flour	5.73	5.73	2.75	62.8
Fermented Dough	5.72	5.06	2.72	64.3
Kisra	5.61	4.95	2.78	63.2
10% Blend:				
Fermented Dough	5.74	4.49	2.69	66.5
Kisra	4.68	4.66	2.58	67.9
15% Blend:				
Fermented Dough	4.76	4.52	2.66	67.8
Kisra	4.88	4.55	2.51	68.1
20% Blend:				
Fermented Dough	4.23	4.19	2.58	68.7
Kisra	4.35	4.40	2.45	68.3
25% Blend:				
Fermented Dough	4.00	3.86	2.44	70.3
Kisra	4.52	3.78	2.35	69.1
30% Blend:				
Fermented Dough	3.94	3.56	2.38	72.4
Kisra	4.03	3.80	2.26	71.5
FAO Reference			2.25	

\* Ratio of essential amino acids(E) to total amino acids (T )  
N

**Table 24. Ratios of Amino Acids as Quality Indices and In Vitro Digestibility of Sorghum Flour from Cultivar 'Dabar' with Indicated Levels of Defatted Peanut Flour**

Sample	Leucine/Isoleucine	Leucine/Lysine	E/T* N	Pepsin-Pancreatin Digest (%)
Sorghum Flour	4.91	6.13	2.65	66.2
Fermented Dough	4.78	5.98	2.62	67.4
Kisra	5.71	6.23	2.65	66.7
10% Blend:				
Fermented Dough	4.75	6.16	2.61	67.0
Kisra	5.10	6.55	2.57	66.7
15% Blend:				
Fermented Dough	3.96	4.11	2.55	68.9
Kisra	4.28	3.88	2.49	67.5
20% Blend:				
Fermented Dough	3.26	2.84	2.45	69.5
Kisra	3.14	3.25	2.43	68.9
25% Blend:				
Fermented Dough	3.36	2.43	2.42	70.3
Kisra	3.12	2.84	2.24	68.9
30% Blend:				
Fermented Dough	2.74	2.18	2.40	72.5
Kisra	2.69	2.72	2.18	69.7
FAO Reference			2.25	

\* Ratio of essential amino acids (E) to total amino acids (T/N)

**Research activities on metal-catalyzed lipid oxidation in spreadable peanut paste (O. F. Agbo, J. C. Anderson, and B. Singh)**

The previous year's report detailed the scope of this activity. The progress to date is herein reported. The purpose of the research is to investigate the effects of metal-catalyzed inducements of lipid oxidation and to evaluate the extent that metals from the steel and cast alloy surfaces of an attrition mill tend to increase the rates of quality deterioration in spreadable peanut pastes.

To determine the roles of metals from the surfaces of the attrition mill on the rates of spreadable peanut paste quality deterioration, peanut pastes were prepared and the progress of rancidity development during an extended period of time was monitored. Metal contents of the peanut pastes will be measured to determine the fraction of metals introduced to the pastes from the surfaces of the attrition mill. The effects of other environmental factors have also been investigated during the process of the storage studies. Work in three phases has been identified as follows:

Phase 1: Preparation of Peanut Paste

Phase 2: Measurement of Rancidity

Phase 3: Analysis of Metals in Peanut Paste

All tasks in both phase 1 and phase 2 have been completed with the bulk of the activity in phase 3. Comprehensive preliminary studies have been done in the phase 3 area.

A 2<sup>(5-1)</sup> fractional factorial experiment consisting of five variables was selected. The variables were milling method (stone slab and plate-type attrition mill), EDTA (with and without sequestrant additions), oxygen (vacuum and atmospheric packaging), light (dark and fluorescent illumination) and temperature (4°C and 45°C). Various treatment combinations of the five variables were randomly assigned to the samples of the peanut pastes. Peanuts (cultivar Florunner No.1, obtained from Gold Kist, Inc.) were roasted at 210 +/- 5°C in a wok pan using an electric heater as the heat source. Ceramic beads, 1/16 in. (1.6 mm) in diameter obtained from Coors Porcelain Co. (Golden, CO), were used as a heat transfer medium in a particle-bed roaster. The ceramic beads were used instead of sand to avoid possible carryover of sand which may affect the aesthetic quality of the finished products and to prevent possible contamination of the product with silicate which may increase the rates of lipid oxidation. The roasting process continued for an estimated residence time of 15 min (starting from the time the temperature of the peanut and the ceramic beads reached 210 +/- 5°C). The testa of the roasted peanuts were winnowed free from the peanut meats and the roasted peanut meats milled using alternately the traditional method and the plate milling method.

Rancidity (as determined by TBARS number) was monitored on a biweekly basis for 127 days on all stored spreadable peanut paste samples using published methods. Peroxide (PO) values were determined using the official method of the American Oil Chemists Society (A.O.C.S., 1978) greatly scaled down to permit determination of peroxide content in 500 +/- 50 mg of oil. The work remaining in this area is organization of the data collected during the storage studies into a logical presentable order of final report.

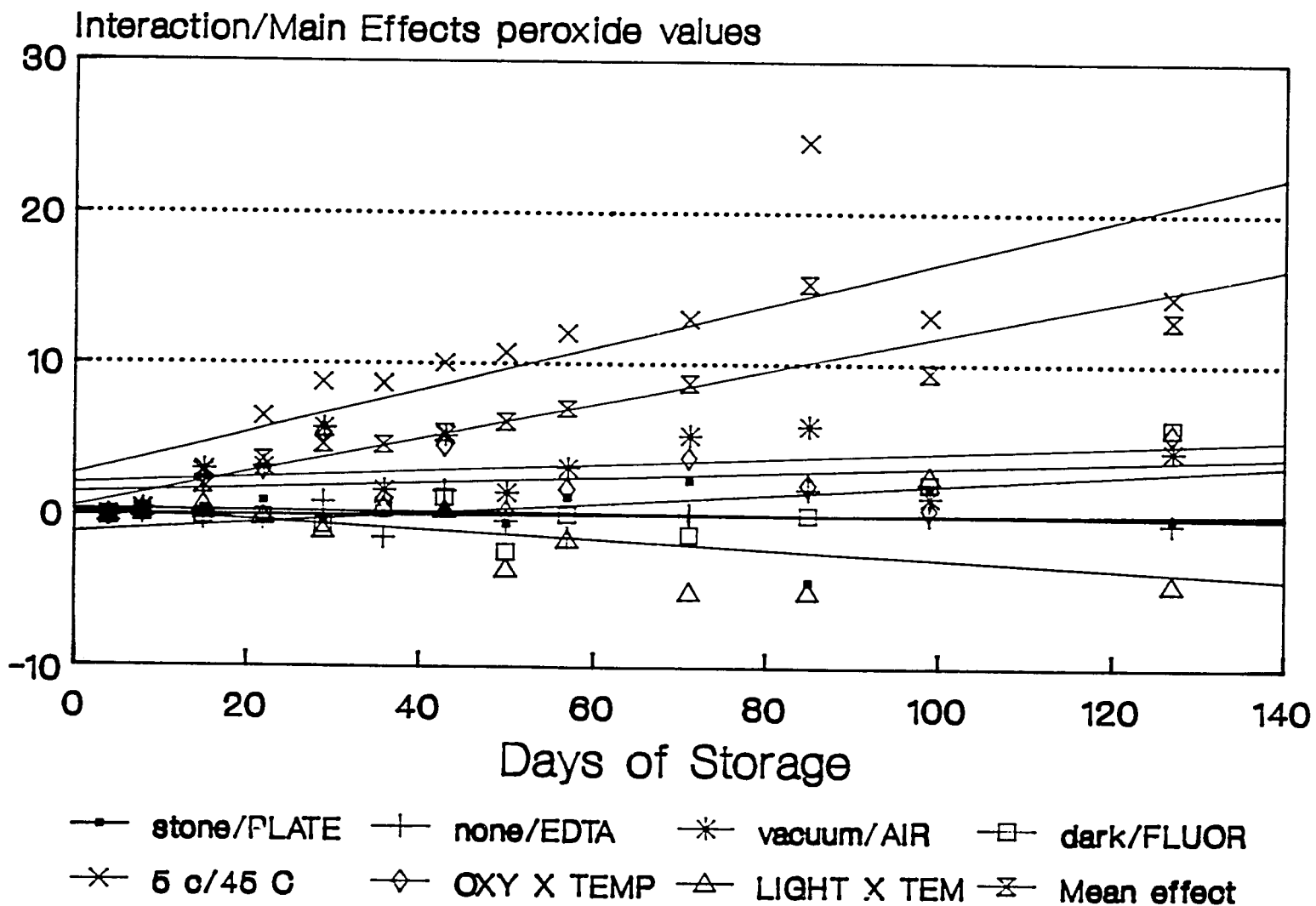
The work in this area is in process. It is our intention to analyze raw peanuts and roasted peanuts milled by stone and plate method for iron, copper and zinc to estimate the fraction of metals introduced in the peanut samples from the surfaces of the attrition mill. We shall determine the metals using the atomic absorption spectrophotometric method as follows: All glassware will be immersed in EDTA (0.5%) for 24 hrs and thoroughly rinsed with de-ionized glass-distilled water to prevent contamination by extraneous heavy metals. A 2.0 g portion of the peanut pastes will be ashed. An aliquot representing approximately 8 ppm of iron and copper will be immediately analyzed using the Perkin-Elmer model 3030 atomic absorption spectrophotometer. Determination of zinc will require analysis of an aliquot representing 0.8 ppm of zinc. Percent absorbance will be recorded by aspirating the samples into the atomic absorption flame with wavelength set at 213.8 nm for zinc, 248.3 nm for iron and 324.8 nm for copper.

Trend analysis of the main effects and the two consistently significant interactions are presented in Fig. 14. The data collected during the 127 days storage study showed that first elevated temperature and second oxygen significantly increased the extent of peroxidation of lipids in peanut paste. Peanut paste processed with a plate-type attrition mill indicated only a slightly positive trend that was not significant. Addition of EDTA (100 ppm) diminished only slightly the extent of lipid oxidation. Exposure of the peanut paste to fluorescent light had a small negative effect in the early stage of the study but it turned positive subsequently. Generally, vacuum-packaged, low-temperature storage treatments appeared to produce the most stable peanut paste regardless of the level of chelator, light or milling method used.

# Interaction/Main Effects - Storage Study

## Milling/Chelator/Oxygen/Light/Temp

Figure 14. Interaction/Main Effects - Storage Study



## Burkina Faso (University of Ougadougou)

In Burkina Faso, peanut constitutes a common food product. (1) It is used as a source of oil. (2) It is directly consumed with or without cooking. (3) It is a constituent of many local recipes: sausages, meats, coucous, etc. (4) It is one of the rent products in Burkina Faso. Only Public Service through the National Office of Agricultural Products Price Assessment (CSPPA) is authorized to import or export it.

Table 25 provides the data on peanut production in Burkina Faso since 1975. Two important interpretations can be obtained from this table: (1) The yield increased for the last three years because of the better prices given to peanut by the National Office of Prices. (2) There are variations in the yields of peanut from one year to another due to drought, diseases and cultural practices. For instance, one of the most common diseases of peanut is rust disease. It can decrease the production from 40 to 50%. Fig. 15 summarizes the situation of this disease in Burkina Faso since 1977. Three geographical zones can be seen. They are linked to water systems in the country. In Burkina Faso, according to the cultivars, peanut crop covers a period from August to October. The problem in postharvest handling and storage results in reduction in quality due to insect infection, mold contamination and mycotoxin contamination. In this first report, chemical components of peanuts grown in Burkina Faso have been determined. This includes cultivars provided by the US collaborators and also the locally grown cultivars.

### SAMPLING

#### Imported cultivars of peanut

So far, CRSP project at ISP has been concerned with the production, entomology and phytopathology of some cultivars of peanuts. They have been tested in three geographical sites: (a) Gampela (b) Niangoloko (c) Saria. These cultivars were harvested in October, 1987. Samples of 800 to 1000 g of peanut were sun dried for a period of 30 days. Then they were stored in polyethylene bags at room temperature (25 - 30 degrees C). These cultivars are listed in Table 26.

#### Local cultivars of peanut

Samples were collected from different parts of the country. In contrast to American cultivars, the local cultivars have a longer growth period and are listed on Table 27. After the local cultivars were collected, each was stored according to the traditional methods of each region.

### PHYSICO-CHEMICAL PARAMETER ASSESSMENT

The samples were analyzed in the Food Control Laboratory of ISP at University of Ouagadougou. For this purpose, each sample was ground in an Osterizer-Galaxie grinder for chemical analyses (Table 28) using standard procedures.

### RESULTS

This work has been completed in collaboration with the CRSP project from which the American cultivars of peanut were obtained. Local cultivars were given by Food Technology Service of Agricultural Ministry. Table 29 shows the results obtained with the samples from the agronomic station of Gampela. In general, these samples had: (1) higher acid value and relatively high moisture contents and (2) relatively low aflatoxin concentrations.

The same cultivars, but produced in Niangoloko, are presented in Table 30. Acidity and water contents were low and the aflatoxin contents were relatively high. Apparently, peanuts from this site were contaminated with *Aspergillus flavus* or other species. Considering the results of samples from Saria, one can see that they are more or less similar to those obtained from Niangoloko (Table 31). The results on the local cultivar samples from Burkina Faso are listed in Table 32. In general, (a) acid value was low, (b) protein and fat contents were higher than that of the U. S. cultivars, and (c) aflatoxin content was also low.

Table 25: Production of Peanuts in Burkina Faso

Period	Area planted (ha)	Yield (metric tons)
1975 - 1976	164,000	87,000
1976 - 1977	137,000	73,000
1977 - 1978	135,000	57,000
1978 - 1979	152,320	74,000
1979 - 1980	153,953	79,000
1980 - 1981	105,677	54,000
1981 - 1982	128,086	78,000
1982 - 1983	148,000	73,000
1983 - 1984	137,000	82,000
1984 - 1985	-	71,000
1985 - 1986	-	127,786
1986 - 1987	-	158,689
1987 - 1988	-	145,857

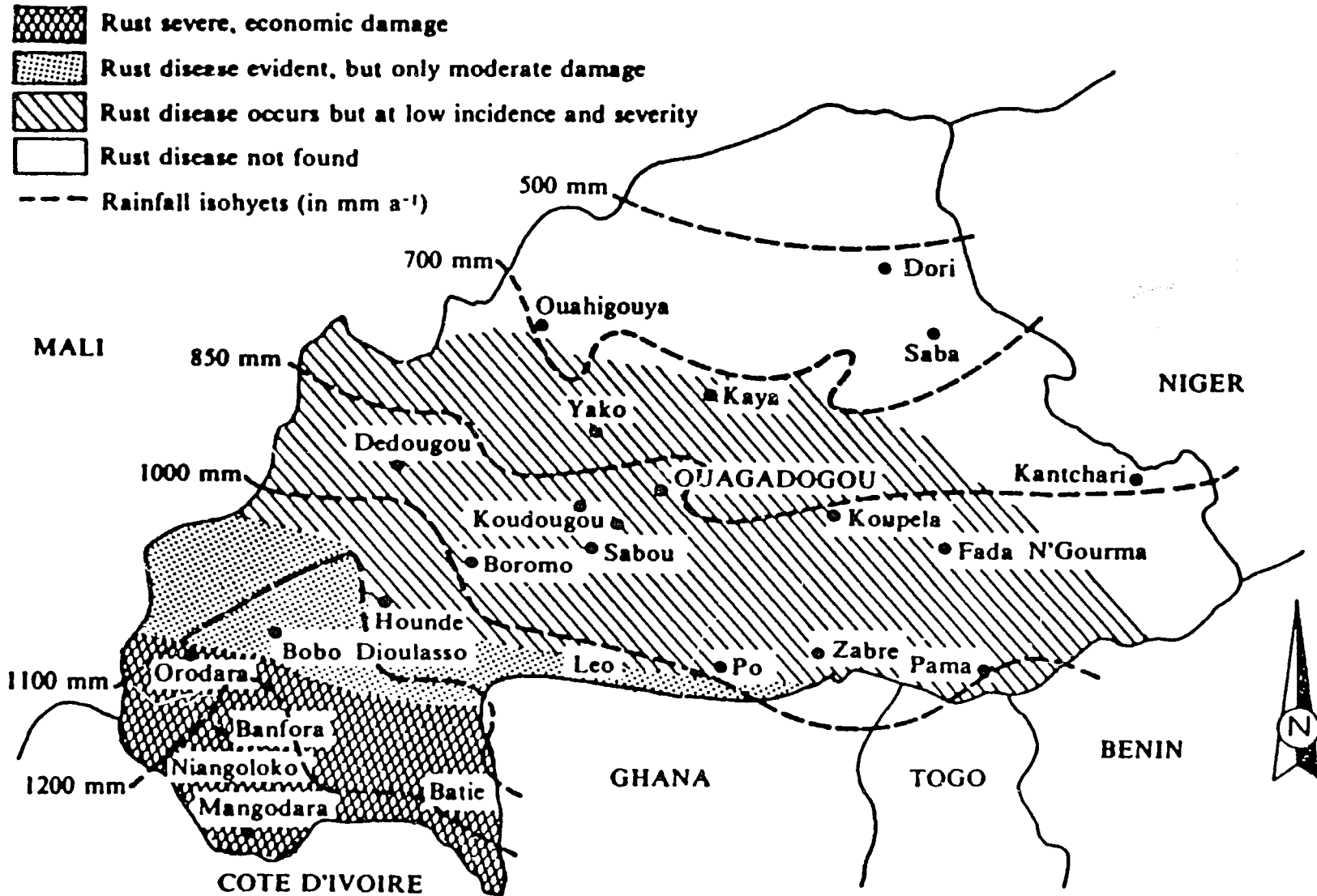


Figure 15. The occurrence and severity of rust disease of groundnut in Burkina Faso



**Table 26: List of American Cultivars of Peanut Studied**

Cultivars	Sample Designation	Type	Time of Maturation (days)
5	TAM NUT 74	Spanish	90
6	TOALSON	Spanish	90
7	PRONTO	Spanish	90
8	SPANCO	Spanish	90
10	N.M. VAL. C	Valencia	90
15	SOUTHERN RUNNER	Virginia	110
16	FLORUNNER	Virginia	110
19	EARLYRUNNER	Virginia	110
31	CBR - R1	Spanish	90
48	UF - 81 24 - 53	Virginia	110

**Table 27: List of Local Cultivars of Peanut Studied**

Sample #	Identification Code	Location
1	R M P 12	Diebougou
2	R M P 12	Niangoloko
3	R M P 91	Diebougou
4	R M P 91	Niangoloko
5	T S 321	Koupela
6	BALOGO	Balogo
7	C N 94 C	Yatenga
8	90	Leo
9	140	Leo
10	59 - 426	Bobo-dioulasso

**Table 28: Methods for Characterization of Different Parameters**

Physico-chemical parameters studied	References of methods used
-Teneur en eau	Normes française (NF) T 60 - 201 du Mars 1968
-Acidité'	N.F. T 60-204, Decembre 1968
-Cendres totales	N.F. V 03-923, Janvier 1967
-Prote'ines brutes	N.F V 18-200, Octobre 1977
-Matières grasses	N.F. V 03-905, Decembre 1973
-Energie métabolisable	FAO: Energy yielding components of food computation of calorie values, May 1947
-Aflatoxin B1	N.F V 18-200, Juin 1980
-Vitamine C	Bessey: J. Assoc. Off. Agric. Chemists 27:537 (1944)

**Table 29: Composition of American Peanuts from Agronomic Station of Gampela**

CULTIVAR (refer to Table 26)	Water content %	Acidity % oleic acid	Ash %	Protein %	Fat %	Total sugar %	Energy kcal/g	Aflatoxin (B1) ppb	Vitamin C mg/100 g
5	4.3	18.8	2.3	21.5	42.5	10.8	4.7	24	6.1
6	4.5	21.9	2.4	23.7	45.2	13.0	5.1	6	6.3
7	3.8	12.7	2.4	18.9	43.6	13.4	4.8	19	6.6
8	4.0	23.9	2.5	14.6	46.3	10.5	4.8	7	6.2
10	4.2	8.1	2.5	16.6	43.3	11.4	4.7	19	7.3
15	4.5	25.0	2.4	20.5	42.2	11.9	4.7	7	6.1
16	4.3	22.9	2.3	13.2	43.2	11.5	4.5	10	6.1
19	3.9	14.4	2.3	13.6	42.4	11.1	4.5	7	5.7
31	4.6	24.5	2.4	23.6	46.9	11.1	5.2	21	6.7
48	3.9	7.5	2.3	18.1	45.2	11.3	4.9	5	6.8

**Table 30: Composition of American Peanuts from Agronomic Station of Niangoloko**

CULTIVAR (refer to Table 26)	Water content %	Acidity % oleic acid	Ash %	Protein %	Fat %	Total sugar %	Energy kcal/g	Aflatoxin (B1) ppb	Vitamin C mg/100 g
5	4.0	1.5	2.6	14.0	44.8	11.3	4.7	15	14.3
6	3.5	2.2	2.9	17.2	39.7	14.0	4.5	11	11.8
7	4.7	1.0	2.9	19.4	43.5	13.6	4.8	11	12.7
8	3.7	-	3.2	17.3	45.5	12.8	4.9	15	15.3
10	3.3	4.2	3.5	12.1	40.2	11.9	4.2	11	10.3
15	3.3	1.2	3.2	19.6	44.3	11.3	4.8	15	12.2
16	3.4	2.4	2.9	23.2	44.1	13.4	5.0	17	14.9
19	3.2	-	2.6	21.4	44.6	-	-	12	13.9
31	3.3	3.3	2.8	22.0	43.6	11.4	4.9	8	11.6
48	3.2	2.2	2.9	22.4	43.1	12.5	4.9	7	13.3

**Table 31: Composition of American Peanuts from Agronomic Station of Sarria**

CULTIVAR (refer to Table 26)	Water content %	Acidity % oleic acid	Ash %	Protein %	Fat %	Total sugar %	Energy kcal/g	Aflatoxin (B1) ppb	Vitamin C mg/100 g
4	3.4	1.4	2.3	13.8	47.9	13.3	5.0	11	8.4
7	3.3	0.8	2.3	19.3	45.2	15.5	5.0	11	8.8
8	3.2	1.6	2.5	18.5	43.4	14.3	4.9	11	9.2
10	3.2	1.7	2.8	21.1	42.0	13.3	4.8	14	9.2
15	3.0	0.7	2.4	16.7	43.3	13.6	4.7	10	9.0

**Table 32: Composition of American Peanuts from Agronomic Cities in the Burkina Faso**

CULTIVAR (refer to Table 26)	Water content %	Acidity % oleic acid	Ash %	Protein %	Fat %	Total sugar %	Energy kcal/g	Aflatoxin (B1) ppb	Vitamin C mg/100 g	Location
RMP 12	3.1	1.2	2.3	29.2	49.8	11.6	5.7	12	8.4	Diebougou
RMP 12	4.9	2.1	2.9	23.2	45.8	15.2	5.2	29	9.0	Niangoloko
RMP 91	3.4	0.7	2.9	26.7	49.5	15.5	5.7	8	9.2	Diebougou
RMP 91	5.2	1.0	2.3	22.2	47.9	13.3	5.3	14	9.0	Niangoloko
TS 321	4.2	0.8	2.3	26.7	46.4	13.4	5.4	8	10.0	Koupela
BALOGO	4.0	1.7	2.2	29.5	46.0	14.0	5.4	10	12.9	Balogo
CN 94C	4.4	1.1	2.3	20.8	46.3	20.5	5.4	11	10.3	Yakenga
90	4.6	2.2	2.5	25.7	46.2	24.3	5.7	11	8.4	Leo
140	4.3	0.8	2.5	14.0	49.0	13.3	5.1	13	9.6	Leo
59 - 426	4.3	2.3	2.7	19.0	46.2	15.2	5.2	12	9.0	Bobo-Dioulass

**Research Plan for 1988-1989**

1. Continue work on evaluation of quality of peanuts grown at various locations in Burkina Faso.
2. Initiate research on quality of peanuts under storage conditions in Burkina Faso: insect infestation, mold contamination, and aflatoxin contamination.
3. Continue work on evaluation of quality of peanut paste available in market places in Burkina Faso: consistency, oil content, rancidity, contamination and storage quality.
4. Initiate collaboration work with entomologist on storage pests and possible modifications to reduce losses in quality of peanuts during storage.
5. Initiate collaborative research on quality of peanuts grown in the U.S. and Burkina Faso.
6. Initiate research on fortification of "Toe" ( a sorghum-based product), a collaborative research plan with Texas A&M (Sorghum-Millet CRSP) and Alabama A&M.
7. Initiate collaborative research with Bean-Cowpea and Peanut CRSP's on possible products (composite cereals—peanuts and cowpeas).
8. Complete research on metal-catalyzed lipid oxidation in peanut pastes.
9. Continue research on characterization of soluble nutrients in leachates from water blanching and steam blanching.
10. Continue work on suitable and affordable roasting methods.
11. Complete research on "Kuli-Kuli", a snack food made up of partially defatted peanut pastes.
12. Develop linkages with research groups in Africa and Asia on quality of and food product aspects of peanuts and other legumes especially through ICRISAT.

## GA/PH/C

## POSTHARVEST HANDLING SYSTEMS FOR THE SMALL PEANUT PRODUCER

University of Georgia  
Manjeet S. Chinnan

Caribbean Agricultural Research and Development Institute  
Brian Cooper, B. K. Rai, J. Grant, M. Rao, and G. Muller

## 1. INTRODUCTION

It is a well documented fact that the quality control of farm commodities begins on the farm and continues through postharvest handling, storage and processing steps until the point of consumption. Improper handling of peanuts after they are harvested can be a primary factor associated with the reduction of market value and decrease in probability of consumption. Another factor affecting the probability of consumption of indigenously grown peanuts is their cost, which in turn is influenced by the cost associated with production, harvesting and postharvest operations.

Some of the original objectives of the project (GA/BCP/CAR) in reference to identifying improved cultivars and limiting cultural practices have been met to a great extent. It was proposed that emphasis be shifted from increasing production to identifying suitable methods for maintaining postharvest quality and to developing approaches for reducing cost associated with harvesting, threshing and post-harvest handling steps.

## 2. MAJOR ACCOMPLISHMENTS

**2.1 Getting the project off the ground.** There was a transition period involved in shifting the emphasis on cultivar evaluation from an earlier project (led by Dr. Craig Kvien) to the current project focusing on postharvest handling operations. In addition there were some organizational and personnel changes in CARDI. However, significant progress has been made towards meeting the project objectives after a slow beginning.

**2.2 Making site visits to all the collaborating CARDI countries.** Trips were made to Antigua, Belize, Jamaica, St. Vincent and Trinidad to collect information on existing peanut production and post-harvest approaches. The trip to Antigua was made in May, 1987, before the project proposal was submitted.

**2.3 Hiring of a Research Engineer.** A research engineer with experience in designing and fabricating equipment suitable for small farms was recruited. He has assisted the US-PI in coordinating the project activities, training host country personnel, and fabricating and modifying some selected post-harvest equipment appropriate for host countries.

**2.4 Modification and testing of an old thresher for small peanut plots.** Two old threshers were acquired from the University of Georgia Coastal Plains Experiment Station, Tifton. One thresher was refurbished, modified to be transported on a three point hitch and operated from a tractor PTO drive, and then after limited testing it was shipped to Jamaica.

**2.5 Fabrication of a small scale sheller.** A hand operated sheller with replaceable screens was fabricated at Georgia, which was further modified to adapt to pedal power operation. Extensive testing on different seed-sized peanuts was done.

**2.6 Hosting a CARDI collaborator and a visiting scientist.** PI from Jamaica spent 10 days in Griffin with Georgia researchers working on various aspects of the project objectives. A research scientist from India, funded by United Nations, began a three month training program at Georgia in May, 1988, making scientific and technical contributions towards CRSP project objectives.

**2.7 Evaluation of drying and storage systems.** Drying and storage systems in all the collaborating countries were evaluated during site visits, and work was planned to monitor those systems at selected locations, including the study of aflatoxin levels.

**2.8 Continuation of varietal testing.** Some work was continued in this area to provide continuity from work initiated in an earlier project.

### 3. EXPECTED IMPACT OF PROJECT

The Caribbean countries can probably maintain a sustained production of peanuts, given a suitable cultivar. However, current methods of harvesting and postharvest handling operations result in high cost and low quality peanuts for the consumer. A production package, emerging from this project, should increase the availability of low cost nutritious foodstuffs to the rural and urban poor through the introduction of more efficient production, harvesting, drying, storage and handling techniques.

### 4. GOAL

The goal is to develop a system-oriented package which will have an impact on the economic conditions of the peanut producers in the Caribbean basin. The major thrust will be in the area of improved methods of postharvest operations. The information developed should help in providing linkages between breeding, farming systems and food technology projects in other regions where CRSP projects are active, particularly in southeast Asia.

### 5. OBJECTIVES

The proposed research is focused on attainable goals during the three-year period beginning July 1, 1987. Specific objectives are as follows:

1. Investigate appropriate technologies for the drying and storage of in-shell peanut, and storage of shelled peanut.
2. Identify and/or develop labor saving devices for production and postharvest operations.
3. Develop strategies for linking Southeast Asia Peanut CRSP projects on breeding, farming systems and food technology.

### 6. ORGANIZATION

- 6.1 U. S. Lead Institution : University of Georgia
  - 6.1.1 Department of Food Science and Technology, Griffin
    - Dr. Manjeet S. Chinnan, Principal Investigator
    - Mr. Tal Oz-Ari, Research Agricultural Engineer I
    - Mr. J.-S. Chern, Computer Programmer III
    - Mr. Glen D. Farrell, Laboratory Technician III
  - 6.1.2 Department of Agricultural Engineering, Griffin
    - Dr. Brahm P. Verma, Professor and Head
    - Mr. Durward Smith, Workshop Manager
  - 6.1.3 Department of Agronomy, Tifton
    - Dr. Craig Kvien
    - Dr. C. Holbrook
  - 6.1.4 Department of Plant Pathology, Tifton
    - Dr. Alex Csinos
    - Dr. Dave Wilson

## 6.2 Host Country Institutions

### 6.2.1 CARDI (Caribbean Agricultural Research and Development Institute)

Dr. Brian Cooper, Principal Investigator (Antigua) and Principal Coordinator for all CARDI locations

Ms. Monica Gordon, Agronomist (Antigua)

Dr. B. K. Rai, Principal Investigator (Belize)

Dr. Janice Reid, Country Representative (Jamaica)

Mr. Joscelyn Grant, Principal Investigator (Jamaica)

Mrs. Barbara Black, Coordinator of CARDI Projects (Jamaica)

Mr. Maurice Taylor, Research Assistant (Jamaica)

Dr. Murali Rao, Principal Investigator (St. Vincent)

Dr. Gordon Muller, Principal Investigator (Trinidad)

### 6.2.2 University of West Indies, St. Augustine, Trinidad

Dr. Clement Sakant, Department of Agricultural Engineering

## 7. APPROACH

a) There are five CARDI countries involved in this project. The first and foremost step relates to making site visits and gathering information on existing practices of the harvesting and postharvest handling techniques in those five countries.

b) Identify mechanical aids for postharvest operations such as threshing and shelling. Adapt the existing equipment or modify and fabricate as necessary at Georgia or in host country for use in host country.

c) Investigate innovative separation techniques for removing foreign material from shelled and in-shell peanuts.

d) Develop strategies for postharvest drying of in-shell peanuts for efficient, low cost drying systems suitable to respective growing regions.

e) Evaluate existing storage methods, and then recommend and implement improved storage techniques. Monitor quality (aflatoxin levels) of peanuts in storage.

f) Provide training and assistance to host country personnel for the development of effective postharvest handling techniques.

g) Make site visits to other peanut growing regions of the world, specifically southeast Asia, for developing strategies for improved postharvest handling methods in those regions.

## 8. ACCOMPLISHMENTS IN DETAIL

### 8.1 GETTING STARTED

The initial task in this project was to make a smooth transition from an earlier project on the farming systems to the current one in the area of postharvest handling systems for the small farmer. In May of 1987, former US Principal investigator Dr. Craig Kvien, who headed the farming systems project, introduced the current US principal investigator to all the principal collaborators of the CARDI. As a result, the current three-year proposal (July 1987 - June 1990) was drafted on postharvest handling aspects of peanut production systems. The initial draft of the proposal was presented to the Technical Committee and then the Board of Peanut CRSP at the 1987 APRES Meeting for their approval before this project could be officially begun. The Board required revision of the proposal, which was so done and submitted to the Technical Committee.

## 8.2 SITE VISITS - INFORMATION GATHERING

After getting an official go ahead, the first order of business was to make site visits to all the host countries associated with the project. The collaborating countries are Antigua, Belize, Jamaica, St. Vincent, and Trinidad. The first trip was taken to Jamaica, St. Vincent and Trinidad in December of 1987. The second trip was taken to Belize in February of 1988. Antigua was not visited during the 1988 fiscal year, because the US-PI had visited Antigua in May 1987 during the initial stages of the project proposal preparation; however, Dr. Brian Cooper, who is the CARDI scientist at Antigua and also serves as the Peanut CRSP project coordinator for all the five host countries accompanied the US-PI during the St. Vincent and Trinidad section of the first trip mentioned above.

## 8.3 ACTIVITIES AT GEORGIA

### 8.3.1 HIRING OF A RESEARCH ENGINEER AT GEORGIA

It was felt necessary to have a full time research engineer to assist the US-PI and coordinate the activities with the host country researchers. In December of 1987 Mr. Tal Oz-Ari was employed, an agricultural engineer with a number of years of experience in fabrication and maintenance of machinery and farm equipment.

After the first round of visits to host countries it was concluded that the workshop facilities at Georgia will be used for doing most of the modifications and the fabrication of any projected related equipment. Mr. Tal Oz-Ari would be the person in charge for these activities. It was also found that Jamaica has potential for having full-fledged workshop facilities at the Lawrence Field Station (LFS), which is about 20 km from the Mona Campus (Kingston) of the University of West Indies. At present the LFS station has an enclosed building with about 2500 sq. ft. floor area but is inadequately furnished with shop equipment, machinery and tools. These facilities could be used as a research and development workshop, when fully equipped, not only to serve the CARDI needs in Jamaica but also other CARDI countries. The US-PI and the research engineer have developed a proposal consisting of three alternative plans to upgrade the workshop in Jamaica. The proposal was submitted to CARDI so that they could find funds from sources other than Peanut CRSP to adequately equip the workshop.

Although the Agricultural Engineering Department of the University of West Indies (UWI) at St. Augustine, Trinidad, has an excellent workshop facilities for any equipment development and fabrication, these resources are not being effectively utilized by CARDI personnel. However, potential exists for cooperation between these two organizations. Plans were developed to seek ways to strengthen the linkages to benefit this peanut project.

### 8.3.2 THRESHER

It was found that one of the limiting factors in peanut production system is the cost associated with threshing. It is estimated that cost of producing one pound of peanuts in Belize is 60 cents, improved threshing and planting equipment can reduce the cost to 40 cents a pound. It was learned from the farmers in Jamaica that up to 20% of the production cost may be associated with threshing. All the threshing in the CARDI countries is presently done manually, where the damaged and the immature pods are discarded at the same time. Mechanical, but low cost, thresher can probably not only reduce the threshing cost but also eliminate the human drudgery involved in manually picking the pods off the vines, one pod at a time.

Two old CeCoCo threshers were obtained from the University of Georgia Coastal Plains Experiment Station, Tifton, with the objective of refurbishing and modifying them for use in the host countries. One of the two threshers, called Thresher-I, was refurbished at Georgia and modified so that it could be operated from a tractor PTO (power-take-off) shaft. Provision was also made so that a 5-8 hp gasoline engine could be mounted, if necessary, to power the machine. Mr. Joscelyn Grant, principal investigator from Jamaica, an agricultural engineer, had significant input in the modification of this

thresher, during the period he was visiting Georgia. Mr. Ishwar K. Garg, a visiting scientist from the Punjab Agricultural University, Ludhiana, India, also provided input in the thresher modification.

Thresher-I was shipped to Jamaica for field testing in FY 1989, on the peanut crop there. The second thresher, called Thresher-II, was saved to be reworked and modified in the FY 1989 for preliminary testing in Georgia and then testing in field in Belize.

### 8.3.3 SHELLER

A hand operated peanut sheller was fabricated in Georgia based on the design concept employed by the CARDI engineers in Jamaica few years back. Two of the known problems with the Jamaican sheller are : one, it is heavy to transport from one location to another; second, it has a fixed, one-size screen which means that it is suitable for only one size pods. The Georgia version was designed so that, first, it could be lifted on a three point hitch on a tractor for easy transportation; second, the screen (through which shelled peanuts pass as a result of the shelling action) was easily replaceable. Several different concepts in designing removable screens were investigated. The Jamaican model is hand operated which requires reciprocating action given to a handle and its continuous operation for extended periods can be very tiring. The Georgia model was further modified so that it could be pedal-powered. Although Mr. Oz-Ari did most of the fabrication, both Mr. J. Grant and Mr. I. K. Garg (visiting scientist) had significant contributions in the design aspects of the device. Preliminary testing of the sheller was conducted by I. K. Garg and Georgia researchers, and the device was found to perform satisfactorily. Additional testing is planned in the next fiscal year before the sheller is shipped to one of the collaborating countries.

### 8.3.4 Drying and Storage

Drying and storage facilities in all the collaborating countries were surveyed by the US-PI during the initial site visits. Status of those facilities and the research activities associated with them are discussed later in this report.

## 8.4 ACTIVITIES IN HOST COUNTRIES

The new direction (shift from production oriented studies, primarily varietal selection and agronomic improvements, to an emphasis on pest harvest operations) took some time to be implemented and new work programs and budgets were not finalized until well into the project year, resulting in some delay in implementing the new thrust. Peanut crop from the varietal work also had to be harvested and evaluated.

### 8.4.1 ANTIGUA

#### 8.4.1.1 VARIETAL SELECTION

A replicated trial with four replications comprising 22 varieties, planted May 11, 1987, was harvested in September. Rainfall during the the trial was heavy during the initial stages, but inadequate during the latter part of the trial. Lime chlorosis symptoms, regularly observed on these soils, were severe in some cultivars, despite application at about two-week intervals of foliar fertilizer containing micronutrients which alleviated the problem temporarily. Two cultivars, Georgia 207-3-4 and Virginia Bunch 67, stood as being tolerant to this chlorosis and remained relatively green and vigorous throughout the trial; they yielded 182% and 159% of the best local control (Tennessee Red). Georgia 207-3-4 and Virginia Bunch 67 were superior in pod and dry seed yield and Georgia 207-3-4 had a better shelling percentage. No cultivar tested proved to be faster maturing than Tennessee Red (97 days). Its early maturity is a major feature of this variety, enabling it to produce a crop despite often unfavorable rainfall distribution, and also to reduce the period for rust and leaf spot buildup. Georgia 207-3-4 and Virginia Bunch 67 are later maturing, 123 and 121 days in this trial.



#### 8.4.1.2 ON FARM DEMONSTRATION

One farmer planted a 1/3 acre (average size for peanut farmers in Antigua) plot of peanuts (Tennessee Red) in an area where peanuts are not normally grown. The crop was planted with CARDI assistance in November and harvested in February. Insufficient seed of Georgia 207-3-4 or Virginia Bunch 67 was available for commercial scale assessment. Yields of approximately 1000 lbs/acre (dry pod) were obtained. Rain showers occurring at time of drying in the field posed some problems for the farmer who had insufficient space for finishing the drying process under cover. He incurred some losses due to storage in poly-propylene mesh bags of pods which had not fully dried.

Problems and techniques for proper and effective drying, storage and testing of harvested peanuts were highlighted by this experience. Training and raising awareness amongst farmers concerning losses and the dangers of aflatoxin production in harvested and stored peanuts are also needed. The involvement of the extension service in this process has begun.

#### 8.4.1.3 THRESHING AIDS

A metal comb, designed and produced in Jamaica was evaluated as an aid to stripping nuts from vines. It was compared with the traditional hand picking procedure using 100 plants for each procedure. Although the comb was slightly faster in removing pods from the plants, additional time had to be spent removing the "pops" and immature pods. This resulted in no saving of time overall. In addition the stripping action require considerably more energy than simple picking. It is unlikely that, even with modifications, this approach is likely to be successful.

The alternative of small scale motorized threshers of 3-5 hp that can be attached to tractor and carried in field shows more promise. Some stationary prototypes of this size were inspected in St. Kitts where they were locally built as part of another project. They did not prove satisfactory as they did not lend to easy transport to the field. Two of these will be shipped in next fiscal year to Antigua for modification and evaluation.

#### 8.4.1.4 DRYING

Peanuts are normally dried in the sun, some farmers posses undercover area where drying can be finished off during inclement weather to minimize spoilage. Any kind of mechanical drying system for peanuts is non-existent in Antigua.

An alternative is to provide some cheap shower proof shelters for finishing the drying process. A prototype wooden frame, 12' by 12', with a woven polyolefin film was constructed to serve as a cheap moveable rain shelter. This could be used in a farmers yard, or any flat open area for drying of peanuts as well as other crops. However, this prototype arrangement is yet to be evaluated.

The Food and Chemistry Labs of the Ministry of Agriculture in Antigua has some experimental solar crop dryers. CARDI has good working relationships with this lab, efforts will be made to strengthen the linkages and utilize the resources.

#### 8.4.1.5 STORAGE

Storage studies were initiated using a variety of materials and conditions. Paper bags, simple polythene bags and high barrier Curlon bags have been used, both in ambient and seed room (air/conditioned and dehumidified) conditions. Monitoring of seed moisture levels, germination percentage and aflatoxin levels are being carried out. Seed was purchased from several farmers to provide for these trials and also to provide a source of seed for additional farmers to whom we wish to introduce peanut as a crop and for those with whom we need to work this coming season on the on farm harvesting, threshing and drying aspects.

## 8.4.2 BELIZE

### 8.4.2.1 VARIETAL TESTING

*TRIAL PLANTED 6/19/1987:* Sixteen cultivars were planted in this trial. The individual plot size was 32.3 m and comprised of three beds, each 7 m long. Cultivar ICGS-27 yielded significantly higher than the presently cultivated Tennessee Red. Cultivars PI 314817 and New Mexico Valencia A had lighter kernels than Tennessee Red and were thus rejected. Based on kernel weight, the rest of the cultivars were divided into two categories: a) 53-56 g/100 kernels, b) 74 g/100 kernels. Three cultivars fell in category 'a' and were selected for further testing. These were ICGS-27 (highest yielder), ICGS-24 (highest oil content) and Kidang (heaviest kernel and red pericarp). Two cultivars belonging to category 'b' were selected for further testing; M-13 (high yield) and Shulamith (high yield and heavy kernels).

*TRIAL PLANTED 7/2/87:* Thirteen varieties were planted. Tifrust-13 and PI 315 608 yielded significantly more than Tennessee Red, had heavier pods and kernels and were resistant to peanut rust. NC 342, Florunner and Southern Runner yielded higher than Tennessee Red but the difference was not significant. These five varieties were selected for further testing. The list of the remaining 8 varieties is : NC- 13, NC-17, Holland Station Runner, Makalu Red, Tiferun, Egret, and Va 81 B.

### 8.4.2.2 VARIETY SELECTION BY FARMERS

A small quantity of seed of four varieties (Kidang, ICGS-24, ICGS-27, and Tifrust-2) was given to three peanut producers' cooperatives in the Cayo District, for planting in June 1987. Based on productivity, large kernel size, heavy pod, smooth shell and favorable report from processing plants, they selected Kidang. The farmers multiplied Kidang seeds by planting in December 1987. In June/July, 1988, 13 ha were planted with this variety.

### 8.4.2.3 GYPSUM APPLICATION

Large seeded varieties are inefficient Ca utilizers and need higher Ca in the fruiting zone. In the early eighties gypsum was imported, for use in peanut, by a fertilizer factory and sold at \$27 per 100 lb; which was considered too expensive. Gypsum deposits were discovered in the peanut growing region. In 1987, farmers were organized to collect gypsum rocks, grind in a hammer mill and use on peanut. This was done quite successfully on Kidang.

### 8.4.2.4 CHEMICAL TREATMENT OF SEEDS FOR IMPROVED GERMINATION

Various combinations of fungicide formulations (involving Captan, Manzate 200, PCNB, Benlate), soil insecticide (Phoxim) and Flordimex (commercial formulation for breaking seed dormancy) were applied to seeds before planting. Summary of results are as follows:

Shulamith had no seed dormancy and application of insecticide or fungicide or a mixture of the two did not adversely affect the germination of the seed.

PI 315 608 had seed dormancy. Only 31% of the seeds germinated in 20 days without chemical treatment.

Application of Flordimex and insecticide slightly improved germination but the insecticide alone did not.

Application of fungicide or when mixed with insecticide improved seed germination over the application of insecticide alone or insecticide + Flordimex.

Application of Flordimax + insecticide + fungicide resulted in maximum germination.

#### 8.4.2.5 PEANUT DRIERS

Peanut drying and storage facilities of four farmers' cooperatives in the Cayo District were visited in February, 1988. Because of long rainy seasons and high humidity conditions it is almost essential to employ mechanical drying systems. The drying facilities visited do employ artificial drying systems for peanuts (fire wood is used as fuel) and other crops. However, there are several problems associated with the current drying arrangements. Air blowers were fabricated locally and were driven by gasoline motors or PTO drives from tractors. No electricity is available to operate the blowers or the burners. The blowers did not give sufficient air at the required static pressure. The alignment of the blower and the source of power needs improvement as misalignment caused frequent breakdowns. Tractor was an expensive and inefficient source of power for peanut driers.

More efficient type of burner using diesel or kerosene as fuel will be identified. Work will be carried out at Georgia for obtaining an appropriate gasoline engine and blower, do proper alignment, and ship it to Belize for testing.

#### 8.4.2.6 STORAGE ROOMS

Each cooperative had a storage room with cement walls and floor and a zinc roof. Unshelled peanut and shelled corn were stored in bags. The problems observed were: (i) rat menace in one room, (ii) disorderly stacking of bags, (iii) the wooden pallets used did not allow for adequate circulation of air under the stacks for cleaning the floor, and (iv) atmospheric humidity was high.

The recommendations were as follows: (i) The doors and windows of the storage rooms should fit tight so that rats could be kept out of the building, (ii) a sufficient number of strong wooden pallets should be made to keep the bags at least 15 cm above the floor, (iii) each stack should be two bags wide and have adequate space all around for inspection and cleaning, (iv) corn and peanuts should be stored in separate stacks, (v) there should be a false roof under the zinc roof to reduce solar heat in the room and the attic should be well ventilated to dissipate the heat, and (vi) the doors of the storage room should be opened only when there was no rainfall and it was bright sunshine.

#### 8.4.3 JAMAICA

Jamaica is the largest peanut producing country in the Caribbean and there was quite a bit of Peanut CRSP activities in the past in conjunction with CARDI. Due to organizational and staff changes and the situation compounded by the changes in the Peanut CRSP project objectives, very little progress was made in the first six months of this project reporting period. Mr. J. Suah, country representative of CARDI in Jamaica, with whom the US-PI had made contacts in May 1987 in Antigua at the project proposal planning meeting, left CARDI before initiating any project related activities in the early period of fiscal year 1988. He was replaced by Dr. Janice Reed, who appointed Mr. Joscelyn Grant, an agricultural engineer as the project leader in Jamaica. This appointment was mutually agreed upon by CARDI and US-PI.

##### 8.4.3.1 VARIETAL EVALUATION WORK

Mr. Maurice Taylor assistant to late Mr. Horace Payne (CARDI breeder at Jamaica) assists Mr. Joscelyn Grant in Peanut CRSP activities. The variety work initiated in early years of Peanut CRSP using ICRISAT seeds had resulted in the selection of ICG 7886 - TIFRUST 2 (V30) by MINAG (Ministry of Agriculture) and CARDI. This variety is now known as CARDI-PAYNE. This seed has been found to be acceptable to local food processors - Frozen Foods and High gate products, for production of peanut butter and brittle.

The CARDI-PAYNE variety was launched in September 1987 and distributed to farmers; 2,144 lbs was distributed to 21 farmers on 27 acres in St. Elizabeth. The farmers have helped in multiplication of seeds. 300 lbs of CARDI-PAYNE was distributed to other CARDI stations and to 23 other individuals. The performance of this new variety in the St. Elizabeth area is monitored by Mr. Taylor.

Some of the seed were bought back from the farmers. The farmers are found to be very enthusiastic about yield and the strength of the stalk which makes harvesting easier. CARDI-PAYNE yields 1,200 lbs/acre compared to Valencia at 800 lbs/acre.

#### 8.4.3.2 PEANUT DRYING AND STORAGE

Peanut drying and storage facilities were surveyed by the CARDI collaborators from Jamaica, Antigua, Belize, Trinidad and Georgia during their joint meeting in Jamaica in May, 1988. Peanuts are hand harvested and dried for 6-10 days in the sun on hard surfaces like the concrete floor in a back yard or the road surface. Peanuts are collected at the end of the day every day during the drying period and spread again on the hard surface (called barbecue floors) next day, weather permitting. Artificial or mechanical drying is almost non-existent. Storage facilities were found to be of poor design for peanut storage. Storage facility of one farmer visited showed inadequate ventilation and a distinct musty smell. Improper storage and long storage periods due to marketing problems resulted in significant losses through insects and rodents. Storage studies to quantify losses from improper storage conditions were planned for next fiscal year. Methods to improve existing storage facilities will be recommended.

#### 8.4.4 St. VINCENT

Drying is done in sun in the field or on the hard surfaces like concrete floor in the backyard. Mechanical drying is non-existent. Several solar dryers, of relatively simple design, were built by the Agricultural Engineer Department at the University of West Indies in Trinidad and distributed to the peanut farmers. Those dryers were never truly accepted by the farmers. Due to change in personnel at the CARDI office in St. Vincent, no significant progress was made towards this project in this reporting period; however, a new dynamic project officer, Dr. Murali Rao, is expected to have significant contributions in the coming year.

#### 8.4.5 TRINIDAD

Dr. Gordon Muller, is again a new collaborator for this CRSP project. No significant project activities took place in Trinidad due to personnel changes.

Peanut production in Trinidad is at a very low level. Increase in peanut production does not seem to be very promising at least in the near future. Some storage studies are planned for the next fiscal year.

Efforts were made to develop strong working relationships with the UWI (University of West Indies) for collaborating with CARDI to meet certain objectives of the Peanut CRSP project. One area where significant progress could be made is identifying a graduate student at the university to work on a research problem associated with the CRSP project.

### 9. TRAINING OUTPUTS

Mr. Joscelyn E. Grant, PI-Jamaica, spent 10 days at Georgia in April of 1988. The primary purpose of his visit was to work with the Georgia research engineer, Tal Oz-Ari, on design concepts and modification of threshers and shellers suitable for the Caribbean region. In addition, he surveyed the equipment and facilities in the agricultural engineering research workshop to gather information for upgrading the workshop facilities in Jamaica.

Mr. I. K. Garg, Research Engineer, from the Punjab Agricultural University, Ludhiana, India was awarded a three-month fellowship by the FAO (Food and Agriculture Organization of the United Nations) under the United Nations Development Program (UNDP) to work at Georgia (with the US-PI) on postharvest handling equipment. Mr. Garg was in Griffin starting May 23, 1988. Mr. Garg brought with him more than 17 years of experience in research and development of farm equipment for the developing nations. While at Georgia he worked on various aspects of designing and testing peanut threshing and shelling equipment.

**10. TRAVEL**

July 1987: Dr. Manjeet Chinnan traveled to Orlando, FL, to participate in the Peanut CRSP meeting held in conjunction with the APRES (American Peanut Research and Education Society) Meeting. Chinnan also presented the project proposal to the Technical Committee and the Board. Three CARDI scientists, Dr. B. K. Rai, Dr. S. Haque and Mr. J. Suah, were also present at the APRES Meeting.

December 1987: Dr. Manjeet Chinnan traveled to Jamaica, St. Vincent and Trinidad. Dr. B. Cooper (PI-Antigua) traveled with Chinnan to St. Vincent and Trinidad.

February 1988: Dr. Manjeet Chinnan traveled to Belize.

April 1988: Mr. J. E. Grant traveled to Griffin, Georgia, for 10 day visit.

May 1988: Dr. Manjeet Chinnan visited Jamaica for a joint meeting with CARDI collaborators (Drs. Cooper, Rai and Muller, and Mr. Grant).

June 1988: Dr. Manjeet Chinnan attended the International Meeting of the American Society of Agricultural Engineers in Rapid City, SD, to develop contacts and collect technical information on peanut postharvest handling systems as related to developing nations.

**11. PLANS FOR 1988-1989**

1. Ship the modified Thresher-I to Jamaica for testing on the peanut farmers' fields.
2. Continue work on the sheller fabricated at Georgia. Test its performance first in Georgia and then ship it to one of the CARDI countries for field testing.
3. Complete refurbishing and modification on the second thresher, Thresher-II; test its performance in Georgia; ship the machine to Belize for testing in the field.
4. Prepare engineering drawings for the thresher and the sheller and have those available to all CARDI scientists.
5. Tal Oz-Ari to visit Jamaica in July/August for 10 to 15 days to assist Jamaica PI in evaluation of thresher (mentioned in item 1); set up storage studies in two different locations - monitor temperature and relative humidity conditions, and development of aflatoxin; and conduct experiments to obtain data on typical drying arrangements.
6. Purchase appropriate blower and gasoline engine and necessary components for aligning blower and the engine; assemble and align the blower and the engine at Georgia; ship it to Belize to be used with an existing dryer there.
7. Tal Oz-Ari to visit Belize in February of 1989 for 7 to 10 days to assist the PI there in setting up storage experiments and test the peanut dryer(s).
8. Dr. Y. Heikal, a Fulbright Scholar from Egypt, will be at Georgia for four months beginning middle of July. He will spend one month on Peanut CRSP related activities. Specifically, he will work on estimating engineering parameters necessary for designing and building dryers for different capacities (ranging from 100 to 20,000 lbs).
9. Two graduate students will be identified to work on the project related objectives; one to work at the University of Georgia and the other at the University of West Indies (UWI). Dr. G. Muller will work closely with Dr. Sakant in recruiting the student at UWI.
10. Variety trial studies will continue at various locations in the Caribbean but only at a very down-scaled level. A blancher will be purchased for evaluating suitability of various cultivars for processing. Belize will coordinate the activities related to the blancher.
11. Storage and drying work will continue at Antigua and will be initiated at St. Vincent. Peanuts in storage will be monitored for aflatoxin and moisture levels.

12. Two small threshers fabricated under an earlier CARDI project will be shipped from St. Kitts to Antigua, where they will be appropriately modified. One will be tested in Antigua. The other one will be shipped to St. Vincent for testing there.
13. Dr. Manjeet Chinnan will make site visits to Thailand and Philippines in May/June 1989 to develop strategies for implementing improved postharvest handling systems and linking Southeast Asia Peanut/CRSP projects on breeding and food technology.