IMPROVING GLOBAL PRODUCTION AND USE OF PEANUT

For Economic Growth, Human Health and Nutrition, and Environmental Sustainability

1996 Annual Report

The Peanut Collaborative Research Support Program

United States Agency for International Development Grant No. DAN-4048-G-00-0041-00

About the Peanut CRSP

Peanut (groundnut) is a crop that enhances sustained, profitable, and environmentally safe use of land with great potential for meeting three food security goals:

- **a widely adapted legume crop that increases available food,**
- **a stable source of cash income for growers, processors, and distributors which increases access to food, and**
- **provides a better nutrient balance for the user.**

The Peanut CRSP (Collaborative Research Support Program) is dedicated to enhancing these potentials through addressing global problems in peanut production and use. Major goals are to generate solutions to current problems and to improve research capabilities that enhance the probability of effectively addressing future problems.

The program involves 10 U.S. universities engaged in collaborative research with institutions in some 12 developing countries that host the Peanut CRSP, seven regional and international organizations, and over 100 scientists, all engaged in priority research and development issues in Semi-Arid Tropical West Africa, Southern Africa, Southeast Asia, South America, and the Caribbean. Additionally, the collaborative nature of the program allows for feedback that provides solutions to producer, processor, and consumer problems in the U.S. The Peanut CRSP communicates information on technologies as they are developed through workshops, networks, training and publications.

The Peanut CRSP was implemented in 1982. It is supported by a grant from the United States Agency for International Development to The University of Georgia with sub-grants to the participating institutions. There is a strong and successful effort to insure complementarity with ICRISAT in program planning, technology development, and communication of information.

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EXECUTIVE SUMMARY for 1995-1996

INTRODUCTION

The Collaborative Research Support Program (CRSP) was created to implement Title XII of the United States Foreign Assistance Act of 1975. The goal of Title XII is to prevent famine and to establish freedom from hunger through land-grant university involvement in international development. To help attain these goals, the Peanut CRSP was established in 1982 to enhance the research capability of developing countries through training and research support, and to support research at U.S. land-grant institutions.

Peanut is grown in most countries, both developing and industrialized, from 40°N to 40°S latitude. Biological and physical environmental constraints to peanut production and use are similar worldwide, which gives great potential for mutually beneficial collaborative research to relieve these constraints.

As world grain production has reached levels consistently near demand, there has been a shift in consumer demand from staple grains to more nutritive, high-protein foods such as peanut. Improved peanut productivity and processing can improve human nutrition and economic development in semi-arid and sub-humid tropical and subtropical environments. Furthermore, peanut cultivation may enhance the agricultural environment as it plays a significant role in increasing the sustainability of agricultural systems.

BENEFITS OF PEANUT PRODUCTION AND USE

Environmental enhancement

- The peanut crop canopy provides nearly full ground cover. Whether grown alone or in sequence with other crops, peanut cultivation reduces exposure of soil to erosion from rainfall or wind.

- The closed peanut canopy also suppresses weeds to reduce weed pressure, especially when peanut is intercropped with a grain crop. In either case, reduced weed pressure reduces need for environmentally-harmful chemical weed control.

Economic growth

- Peanut provides a source of cash income for small-holder farmers, and rural and urban processors in developing countries.

- Biological N fixation by peanut reduces dependence on purchased N fertilizers.

- Often, the peanut crop is processed within the village or country that produces the crop. Thus, peanut production stimulates local food processing industries and adds value to the crop.

- Weed-controlling attributes of the peanut canopy reduce the cost needed for labor-intensive hand weeding or for chemicals to control weeds.

- Peanut is a versatile crop that provides growers with many options to spread risk. With a short growing season, peanut fits well as a cash crop within a large range of cropping systems. It may be grown as monoculture where the rainy season is short, in sequence with grain crops, or beneath a long-lived orchard crop such as banana or coconut.

Improved human health and nutrition

- Peanut has special potential as a famine prevention crop. It may be planted late in a rainy season if the previous crop has failed.

- At 25% protein and 45% oil, peanut provides an inexpensive, high-protein, high-energy food for humans and livestock. It is one of the most nutritive crops available as a complement to cereal grains.

- Peanut supplies a high-quality, healthy vegetable oil for cooking. Supply of cooking oils is often inadequate in developing countries.

- Peanut helps to ensure food security ensuring a consistent supply of food to subsistence farmers and to village and urban consumers who may purchase peanut for food and/or cooking oil.

CONSTRAINTS ASSOCIATED WITH PEANUT PRODUCTION, PROCESSING, AND USE

While peanut offers many benefits to growers, processors, and consumers, there are still several important constraints associated with peanut production and use. The Peanut CRSP was started in 1982 to address a set of global constraints to sustainable peanut production and use. Those constraints served as the initial basis for planning and organizing the Peanut CRSP in 1980 to 1982. Based on past Peanut CRSP accomplishments and the assessment of the External Evaluation Panel in 1989, the following constraints associated with peanut production were confirmed to be valid for the current 1990 to 1995 phase of the program.

Environmental

- Dependance on chemicals to control insect pests and diseases.

- Inadequate diversity in cultivated peanut germplasm grown in developing countries and the U.S.

Socioeconomic

- Low productivity or yield loss from disease, drought, insects, and nematodes.

- Local resource management situations may prevent efficient production and use.

- Insufficient local food supply or inadequate food technology prevents growers, processors, or consumers from exploiting the full potential of peanut because it is not considered as a primary food source.

- Information not available to potential beneficiaries of new peanut production and use technologies.

- Economic losses resulting from mycotoxin contamination.

Health and nutrition

- Health hazard from mycotoxins in contaminated peanut.

- Methods needed to incorporate peanut and peanut products into safe and nutritious processed foods.

Research capacity and tools

- Inadequate numbers of trained research and support personnel in host countries.

- Research tools using molecular biology are not readily applicable to peanut. These tools enable efficient and effective germplasm improvement with respect to resistance to pests and diseases, tolerance to abiotic stresses, seed quality and quantity, and adaptation to growing season. Thus, molecular genetic tools will enhance our capacity to overcome constraints related to environment, economic growth, and human health and nutrition.

In short, the Peanut CRSP enhances the potential of peanut as a cash crop for human food and animal feed in developing countries and the United States. The Peanut CRSP contributes to increasing rural incomes, sustained productivity of agricultural land, and improved health and nutrition of peanut consumers. Furthermore, the Peanut CRSP contributes to enhancing the research capacity of developing country institutions. Collaborative research on peanut is producing new and improved technology that contributes to food security and improves the well-being of people in developing countries and the United States.

THE GLOBAL PLAN

Peanut is a global crop. The crop is widely distributed and important to both developing and industrialized countries. Biological and physical constraints to peanut production are similar worldwide. Research is needed to develop technologies that relieve constraints to peanut production and use, particularly in developing countries with marginal food and protein supply.

For a global crop with global research needs, the Peanut CRSP can catalyze the collaborative efforts of researchers and administrators from diverse groups, including developing countries, U.S. institutions, international agricultural research centers, non-governmental organizations, and donor agencies. The synergism of this collaboration multiplies the impact of Peanut CRSP accomplishments.

Global thrusts

Sustainable production and delivery of food for human consumption are still primary problems in the developing countries. Based on the potential contribution of peanut to sustainable, nutritious food production, the Peanut CRSP has three global thrusts:

1. To develop sustainable agricultural production and food delivery systems that are profitable, environmentally-sound, and relieve important constraints to peanut production and use.

2. To resolve resource management situations that restrict appropriate research or diminish efficiency of systems for peanut production and use.

3. To communicate research outputs to beneficiaries in developing and industrialized countries.

Peanut CRSP benefits and beneficiaries

The benefits of Peanut CRSP collaborative research are adequate quantities of affordable, healthy, nutritious food for rural and urban consumers, increased incomes for peanut growers and processors, and environmentally-sound production and processing systems. Beyond these immediate benefits of improved peanut technologies, community and national benefits ensue from greater profitability of the agricultural sector and a healthier population.

Developing country beneficiaries

- Small-holder farmers, including rural women
- Food processors
- Rural and urban consumers

United States beneficiaries

- Peanut growers
- Processors, marketers, exporters
- Rural and urban consumers

Research strengthening

Progress toward accomplishment of our three global thrusts depends on improved capacity for collaborative research. In host country institutions, the Peanut CRSP trains research and support personnel, and provides critical research equipment. Training has emphasized degree and short-term, non-degree training at host country and U.S. institutions. In both host country and U.S. institutions, the Peanut CRSP supports research activities and facilitates communication among researchers.

Cooperation and coordination

While the Peanut CRSP is the only international program that focuses solely on peanut, there are other research organizations that conduct some peanut research. Since its inception, the Peanut CRSP has enhanced research and technology transfer through cooperative relationships with other international organizations. These relationships enhance cooperative research planning to avoid redundancy across programs, and joint conduct of workshops and symposia to communicate research results. Some organizations with which the Peanut CRSP cooperates are:

- ICRISAT — the International Crops Research Institute for the Semi-Arid Tropics
- ISC — the ICRISAT Sahelian Center (ISC)
- CIRAD-CA — the French Center for International Agricultural Research and Development, Annual Crops Program

SELECTED MAJOR ACCOMPLISHMENTS -- July 1995 through September 1996

Environmental Enhancement

- In the Philippines, reduced funding in the final year of the integrated pest management (IPM) project has limited the overall program and objectives were limited to continued host-plant resistance evaluations and IPM systems evaluation. The overall research program in the Philippines has made outstanding progress in evaluation host-plant resistance, biological control, alternative management strategies, developing economic thresholds, and pest biology. The progress seen in the host plant resistance work has developed an excellent data base evaluation under Filipino production systems. The principal investigators have taken a critical step forward in the final deployment of IPM practices and are testing the integration of this information into IMP programs in the various production regions. Study sites have included the Cagayan Valley, Pampanga, and Mandaue City. These trials have shown that IPM is a viable alternative to standard “farmer managed” plots and conventional insecticide use is often avoided. These studies have looked at current Seed board recommended varieties and promising cultivars used in conjunction with classical biological control programs.

- In the Philippines, biological materials such as Bacillus thuringinensis have proven very effective against a complex of defoliators. These studies and those on the effects of intercropping and fertilizer use have contributed greatly to the development of effective IPM programs. The continuation of studies validating the use of biological control programs using Thichogramma sp. and a microbial insecticide Bacillus thuringiensis show promise for future wide-scale implementation. Biological control studies have shown practicality and profitability using an integrated program in IPM evaluation trials.

- In Thailand, research continued to place primary emphasis on the evaluation of germplasm for insect resistance at both the Department of Agriculture and Khon Kaen University. Studies have focused on the development of a variety of control strategies, including host plant resistance, for IPM programs. Since some farmers use insecticides, on objective included basic insecticide evaluations to improve the timing of application to increase yield and look at the total integrated program using host plant resistance as well as cultural and biological control methodologies. Studies on the relationship of virus incidence and transmission by insect vectors has been slow, but making steady progress. These studies enhance our understanding of the dispersal of several insect pests and the risk of disease transmission.

- More recently studies in Thailand have included research investigating relationship of pod-feeding insect pests and the incidence of aflatoxin. Recent surveys have documented the widespread nature and seriousness of soil insect pests of peanuts in Southeast Asia, therefore this work is critical and long overdue in developing a complete understanding of crop loss assessment. However, research on soil insects is tedious, labor intensive, and slow to provide usable results. It is also important in future aflatoxin elimination management strategies.

- In North Carolina, emphasis continues on the development of a sustainable agricultural system for peanuts in the U.S. Increased effort to minimize preventive pesticide use and to integrate biological and cultural control practices have made significant progress. Coordination with growers organizations and NGO's have established a working relationship for enhanced on-farm validation of this research. The validation of forecast models for the lesser cornstalk borer and spider mites has proven to be an invaluable tool for pest management. Evaluations of pesticide interactions are increasing our understanding to reduce overall pesticide dependence, minimize the threat from tomato spotted wilt virus, and minimize secondary pest outbreaks.

Economic Growth

- "Tamrun 96" formerly identified as Tx896100, a new runner-type peanut adapted for production in the Southwest U.S. peanut production region was released in the late spring of 1996. Seed increases made in 1995 and 1996 brought the total supply to approximately 59 tons which will be used and sold by the Texas A&M University Foundation Seed Services for multiplication and Registered seed production. The principal advantages of the new variety included partial resistance to tomato spotted wilt virus, partial resistance to Sclerotium rolfsii and some other soil-borne diseases, high yield, and good seed size.

- Five runner-type breeding lines with good yields in multiple location trails in Texas and Oklahoma and low relative damage from sclerotinia blight (caused by Sclerotinia minor) in a 1995 disease nursery were selected for pure seed increases of 0.1 to 1.0 acres each.

Shelling, sizing, and quality will be evaluated along with 1996 multiple location, two-state test data for decisions regarding preparation of proposals for release.

- Second year data confirmed the repeatability of differences between genetically high versus low oleic acid Spanish F2 derived populations grown at diverse locations in Texas at altitudes ranging from 370 to 3300 feet. In general, however, the O:L ratios were lower on the Southern High Plains than in South Texas.

- In Texas runner-type segregates with improved oil chemistry and sclerotinia blight and/or spotted wilt virus resistance have been identified and employed in a back crossing program.

- In North Carolina, NC12C peanut cultivar was released in 1996. It is resistant to Cylindrocladium black rot and was tested as breeding line N90013E. Registered seed was producted in the 1996 growing season; certified seed will be producted in 1997 and the cultivar will be in the hands of peanut growers in 1998.

- In North Carolina germplasm release was planned for several genotypes derived from interspecific corsses and possessing resistence to early leafspot (Cercospera arachidicola), root-knot nematodes (meloidogyne incognita), or insects (Helicoverpazer, Empoasca fabae, and Diabrotica undecimpunctata).

- The Philippine seedboard variety/cultivar PSB Pn1, tested as UPL Pn 10, released in 1992 and marketed as 'Biyaya 10', was multiplied and distributed to groweres in the Province of Isabela where it was well accepted by local growers. Biyaya 10 yielded significantly more peanuts than the commercial variety BPI Pn9 (2.44 t/ha vs. 1.95 t/ha) in pilot tests.

- Three new entries were included in the National Cooperative Test conducted at nine locations across the Philippines in May 1996. In trials performed at the Institute of Plant Breeding, all three have produced greater yields, larger seeds, and improved insect resistance compared with Biyaya 10.

- New studies in North Carolina indicate that tomato spotted wilt virus can over-winter in thrips and be transmitted to host plants immediately in the spring. This research helps determine virus/vector relationships with the increasing threat from tomato spotted wilt virus in peanuts which is vectored by these thrips. studies looking at cultivar difference in the incidence of this virus indicate commercially available cultivars such as NCV-11 have less tomato spotted wilt virus that other cultivars such as NC-9. These studies seek to determine if these differences are the result of inherent tolerance of resistance to the virus by the plant or some effect on thrips feeding behavior by different cultivars. Upon completion, these studies will dramatically improve our understanding of the vector and help to manage this disease. These studies complement past vector/virus relationship work in Thailand. The host plant resistance work has been further expanded and has already proven beneficial in identifying resistant germplasm for both foliar and soil insect pests. In addition, this research has revealed rather dramatic differences in the incidence of tomato spotted wilt virus among the various genotypes.

- In Thailand, a comprehensive package for virus control of peanut in Asia has been developed (S. Wongkaew - in press). The basic components of this package incorporates plant resistance, use of virus free seed and cultural practices such as adjustment of sowing date, using high plant density, and intercropping peanut with fast growing non-host crops.

- In Nigeria, efforts continue to develop and release peanut lines resistant to rosette virus. Progress is being made in increased yields of some lines. Some such as Samnut 21 (MDR-8-19) has multiple resistance to rosette, rust and leafspot. The program continues to incorporate drought tolerance and rosette resistance into lines with 90-110 day maturity.

- In Belize, a socioeconomic study was implemented. The questionnaire was finalized based on the instrument developed for the Jamaica study, and the field data was collected during the year. Efforts continued for activities in the area of maintaining post harvest quality and reducing post-production cost through improved post-harvest handling operations including harvesting, threshing/stripping, shelling and storage.

- In Jamaica the main emphasis was on conducting training sessions for consumer taste panels and aflatoxin monitoring in peanut and peanut products. Dr. Margaret Hinds traveled to Jamaica for one week to conduct the training sessions. CARDI has been working very closely with UFMC (United Farmers Multi-purpose Cooperative), and has made significant progress in training of personnel and utilization of post-harvest handling equipment developed or obtained through the project.

Improved Human Health and Nutrition

Mycotoxin reduction:

- In Ghana, a nation-wide study was conducted to determine the extent of contamination of stored peanut by fungi, especially Aspergillus spp. and the aflatoxin levels associated with such seed. The effects of certain plant extracts on aflatoxin synthesis was also studied using a novel microbial assay involving the norsolorinic acid (NOR) mutant of Aspergillus parasiticus Speare. A final publication on the work entitled "High incidence of Aspergillus flavus and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis" is in press, Mycopathologia, 1996.

- Continued cooperative studies with Dr. Thomas H. Adams (Department of Biology, Texas A&M University) have resulted in identification of the entire sterigmatocystin (ST) pathway of Aspergillus nidulans. Evidence show that the sterigmatocystin pathway is functionally equivalent to the aflatoxin (AF) pathway in A. Flavus and A. parasiticus and that this ST/AF toxin pathway is conserved as a cluster of ST/AF genes. Most importantly, the regulatory gene, aflR, is found to regulate both the sterigmatocystin and the aflatoxin pathway in all three Aspergillus sppl Recent progress has identified the likely site of AflR binding to other genes in the ST/AF cluster. We have also found that external pH regulates the biosynthesis of ST/AF and it is likely that the Aspergillus pH regulator, PacC, has a major effect on AF/ST biosynthesis.

- Collaborative work with Dr. Olin Smith and graduate student Yolanda Lopez (TX/BCP) have demonstrated that the use of the norsolorinic acid mutant (re color) may give superior results than a wild-type aflatoxin producing strain of Aspergillus in analyzing aflatoxin contamination of peanuts. A publication has been submitted to Crop Science.

Food processing

- In Thailand, a peanut-based cereal bar formulation was optimized to achieve high nutritional value (10%RDA for adults) and consumer acceptability.

- Products containing fermented (peanut tempeh) were developed and tested for consumer acceptability in Nongno Village, Kud-Jub District, Udonthaneee Province, Thailand.

- A mosquito repellant product made from peanut hulls and other natural materials, including bergamot, was successfully developed and tested in Thailand.

- At the Visayas State College of Agriculture, Letye, Philippines, issues of training, economic benefits, market distribution schemes, material sourcing and packaging technologies associated with peanut product processing by women's cooperative groups were evaluated. Enhancement of quality of two peanut products (Lady button and Lady Finger) was achieved using a response surface methodology. The project expanded the number of adopters by introducing peanut-enriched products to the Bubon Integrated Root Crop Processing Cooperative, the San Agustin Rural Improvement Club and a private entrepreneur.

- In Georgia, a honey-peanut butter formulation was developed and optimized for consumer acceptability.

- In Georgia, the kinetics of drying peanuts in terms of energy cost were studied. A simulation program (PNUTDRY) is valid in predicting drying conditions and can be used in the design of peanut dryers of desired capacity with optimum drying conditions.

- In Burkina Faso, isolates from Allium sativum successfully inhibited aflatoxin production by Aspergillus flavus fungi in peanuts. This technology should be useful to protecting peanuts in storage and should be adaptable to village/home level use.

- In Ghana, a production survey showed that most operations in production, harvesting and threshing were manually done, except for sometimes use of tractors in land preparation. Storage prior to market sometimes was as long as nine months. There were 35% of the farmers who did not sort peanuts before marketing to improve prices received. No farmers were cognizant of aflatoxin or in problems. Major problems cited were: unreliable rainfall, poor soil fertility, lack of mechanization for land preparation, harvesting, threshing, and shelling which resulted in high labor costs. All farmers interviewed were willing to adopt new equipment for peanut production if offered to them on a hire-purchase bais.

- In Ghana, a nationwide survey in all ten Regions was conducted to describe the traditional peanut butter preparation and marketing processes. Aflatoxin above the WHO/FAO/UNICEF maximum level for protein supplements of 30 ppb was found in the majority of samples. Samples from the northern areas had lower levels of aflatoxin.

- In Alabama, studies on proximate composition and rheological properties of peanut-fortified corn flour and dough indicate a considerable improvement on dough spreadability and shelf-life with supplementation up to 30% with defatted peanut flour. This information shows that protein enrichment with peanut flour is possible in products containing corn flour.

Molecular Genetics

- In Georgia, fertile transgenic plants of peanut cv. New Mexico Valencia A were produced using an Agrobacterium-mediated transformation system. Laboratory and greenhouse tests on the transgenic plants confirmed the stable integration of the foreign genes into the plant genome. Seed have been produced of transformed lines for resistance to tomato spotted wilt virus, bud necrosis virus, peanut chlorotic streak virus, and peanut stripe virus. Field tests to

determine the degree of resistance to invasion of the infectious virus are currently underway for tomato spotted wilt virus and peanut stripe virus.

- In North Carolina, numerous transgenic lines with the coat protein gene from Peanut Strip Virus have been recovered. Efficacy testing has identified several lines with greatly enhanced resistance against the virus. Transgenic plants are fertile, and the trait for virus resistance is transmitted to self progeny.

Manpower Development

- Graduate Student Training

  In U.S. Institutions

  One host country student completed an M.S. program with CRSP support.

  One U.S. student completed a Ph.D. program with partial CRSP support.

  Four non-host country students completed Ph.D. Programs with partial CRSP support.

  One non-host country student completed an M.S. program with partial CRSP support

  In Host Country Institutions

  Three students completed M.S. degrees in a host country University with CRSP support.

- Seven U.S. collaborators traveled to host countries and provided various types of research training.

- Three Host Country collaborators have come to the U.S. for short-term training.

COMMUNICATIONS

Workshops

- West Africa - The Peanut CRSP, ICRISAT/Sahelian Center, and CIRAD-CA/France co-sponsored the biennial West African Regional Workshop in Accra, Ghana November 18-22, 1996.

- Africa - A two-week training course on viruses infecting peanut and other legumes was held in Pretoria, South Africa during March 1996. The training was partially supported by the Peanut CRSP in cooperation with ICRISAT. Participants were from 10 African countries. Dr. J. W. Demski was an instructor representing the Peanut CRSP. In conjunction with the Workshop, an International Virus Working Group Meeting was held.

- Jamaica - Dr. Margaret Hinds traveled to Jamaica from June 2-8, 1996 to hold a training session for aflatoxin monitoring, and advising local processors to improve quality of their peanut products. Two Workshops (one in Kingston and one in St. Elizabeth) were held for

aflatoxin detection and monitoring training, with a total of 32 persons trained. Processors in three regions were trained.

- Belize and Jamaica - Based on Peanut CRSP technology and information, CARDI/Belize personnel provided training on production and postharvest handling of peanuts to Extension Officers of the Ministry of Agriculture and of the Belize Federation of Agricultural Cooperatives. Similarly in Jamaica, Ministry of Agriculture and CARDI staff provide training to farmers producing peanut.

- Thailand - A Workshop in Training the Trainers on the topic of "Quality Evaluation and Utilization of Food Legumes" was held at Kasetsart University from April 22 to May 24, 1996. The Workshop was attended by 13 participants from six countries: Tanzania (2), Bhutan (2), Sierra Leone (2), Laos (2), Republic of Guinea (1), and Thailand (4). All participants were government officers in work related to legume utilization. Proceedings were distributed at the Workshop. Many examples given during the training program were peanut products developed by the Peanut CRSP project in Thailand. Cooperation with the Department of Technical and Economic Cooperation, Office of Prime Minister under the Thai-AID project facilitated the training course. This training course will be conducted annually and will result in a large impact on processing of legumes as a whole and the nutritional well-being of consumers throughout Southeast Asia as well as in other developing countries in the world in future years.

- Thai Peanut CRSP researchers continued to cooperate with home economists from the Department of Agriculture Extension, Ministry of Agriculture to transfer technology of ground roasted peanut, hot sand roasted peanut and coated peanut processing to housewives in the Kud-Jub District, Udorn-thanee Province. housewives were very receptive and, at the present, they produce and market these products in the area. This site has serve as a model for some other villagers, especially housewives in the Northeastern Thailand and in Laos P.D. R., to develop processing technologies for other Agricultural products.

- Philippines - A two-day Workshop on Peanut Processing was held at the Institute of Food Science and Technology (UPLB). Trainers were Dr. Ricardo R. del Rosario and Ms. Olivia M. del Rosario.

- Philippines - A Seminar/Workshop on Aflatoxin Control for the Food Industry was held May 13-14, 1996 at the Institute of Food Science and Technology (UPLB). The Seminar was co-sponsored by the Institute and the Peanut CRSP-UGA Food Technology Project in cooperation with the Bureau of food and Drugs, Department of Health, and the Food and Nutrition Research Institute, Department of Science and Technology. Of the 20 participants in the Seminar, 13 were from the food industry, one was from a NGO in Mindanao, and six from the academic area. Dr. Ricardo Rosario, Reynaldo Mabesa, and Robert Brackett (University of Georgia) presented lectures.

PEANUT CRSP ADMINISTRATION AND MANAGEMENT

The Peanut CRSP Management Group includes: the Management Entity at the University of Georgia with a Program Director, Assistant Program Director, Administrative & Senior Secretaries, and a part-time Accountant; the Board of Directors with representatives from Alabama A&M University, The University of Georgia, North Carolina State University, Texas A&M University, and ICRISAT; the Technical Committee with representatives from the above four universities and rotating representation from host countries; an External Evaluation Panel with seven members; an AID/R&D/AGR Program Manager; and a BIFADEC liaison.

Management Entity

The Management Entity is responsible to US AID and to the participating U.S. and host country institutions for fiscal and programmatic activities.

Activities:

- Provided logistical and managerial support to Investigators, including program planning, travel clearance, and equipment purchase.

- Prepared necessary reports including; Annual Report for 1995, quarterly reports, quarterly Newsletter, and Annual Work Plans and Budgets.

- Continued the publication of the Arachis International Newsletter in cooperation with ICRISAT.

- Arranged Board of Directors and Technical Committee Meetings.

- Arranged and participated in Annual Peanut CRSP Meeting in Charlotte, NC, July 14, 1995 with the American Peanut Research and Education Society Meeting July 11-14.

- Participated in CRSP Council activities to promote all CRSPs, to foster cooperation among CRSPs, and to seek inter-CRSP research opportunities.

- Coordinated CRSP contributions to the International Arachis Newsletter.

- Primary objective of the Peanut CRSP Meeting on July 14 was to discuss with the present and interested Peanut CRSP researchers the process of extending or writing a new grant. Discussed that the process will be guided by an Expert Panel of 8-10 people and the CRSP will be opened to any interested participant.

- Organized and conducted all meetings of the Expert Panel.

- Prepared draft of a five-year proposal, presented to AID and was approved for the new grant.

- Office Staff - Dr. David G. Cummins continues as Program Director, and Mrs. Grace Hutto as Administrative Secretary. Dr. Keith Ingram, Associate Program Director assumed a tenured faculty position in the Department of Crop and Soil Science, University of Georgia, Griffin Campus in October 1995. Dr. Jonathan H. Williams, ICRISAT-Sahelian Center Peanut Physiologist served as Associate Program Director while on Sabbatical Leave from ICRISAT from October 1995 to July 1996 at which time he was employed by the Peanut CRSP as Associate Director. Mrs. Amy King serves as a Senior Secretary, employed half-time Peanut

CRSP and half-time SANREM CRSP. Mrs. Gina Thomas, Accountant, UGA/Athens, Agricultural Business Office, serves as part time Accountant for the Peanut CRSP.

Board of Directors

The Board is the policy making group in the CRSP and is responsible for establishing the general directions for the CRSP as carried out by the Management Entity. The Board also acts on recommendations from the Technical Committee on budgets, programs, and other CRSP concerns.

Activities:

- Chairman met in Griffin, GA with the Expert Panel on September 11-13, 1995 to discuss and develop the framework for a five-year extension plan.

- Chairman met in Atlanta, GA November 14-17, 1995 with the Expert Panel during the review of the pre-proposals.

- Chairman met in Griffin, GA January 25-27, 1997 with the Expert Panel during the review of the full-proposals.

- Chairman participated in the proposal presentation to AID in Washington, DC April 23-24, 1996.

- Dr. Onuma Okezie, Alabama A&M University continued as Chair for the second year of a two-year term. Dr. Ed Kanemasu, University of Georgia, continued as Vice-Chair.

Technical Committee

The Technical Committee is responsible for formulating program and budget plans for recommendation to the Board. They are also responsible for internal evaluations of the program and advising the Management Entity and Board of any concerns.

Activities:

- The Chairman met with the Expert Panel in Griffin, GA September 11-13, 1995 to develop the framework for the new five year plan.

- The Chairman met with the group in Washington, DC and participated in the presentation of the five-year grant plan to USAID.

- Dr. Tom Isleib, North Carolina State University is Chair and Dr. Manjeet Chinnan, The University of Georgia, is vice-Chair.

External Evaluation Panel

The External Evaluation Panel is designed to give an unbiased view of how the CRSP is progressing toward meeting its objectives. The present EEP members follow.

Dr. Bo Bengtsson, Professor in International Crop Production Science, Swedish, University of Agricultural Sciences, Uppsala, Sweden (formerly Director General of the Swedish Agency for Research Cooperation with Developing Countries and presently Chairman , CIFOR Board of Trustees), research management, crop production.

Dr. John Cherry, Director, USDA/ARS, Eastern Regional Research Center, Philadelphia, PA, food technology, research management.

Dr. Milton Coughenour, Professor of Rural Sociology, University of Kentucky, Lexington, KY, sociological change, technological change, international agricultural development.

Dr. David Hsi, Emeritus Professor of Plant Pathology, New Mexico State University, Albuquerque, NM, peanut pathology, peanut crop management systems, past-President (1982-83) American Peanut Research and Education Society.

Dr. Robert Schilling, Senior Scientist, CIRAD-CA, Montpelier, France, peanut production and use systems/West Africa, research management.

Dr. Joseph Smartt, Reader in Biology, The University of Southampton, UK, genetics of peanut, peanut production and use systems/Southern Africa.

Dr. Handy Williamson, Jr., Professor and Head of Agricultural Economics and Rural Sociology, University of Tennessee, Knoxville, TN, economic and rural development, research management, impact analysis.

- The EEP completed their intensive review in April 1995. The Report was assembled in final form in July 1995.

- Dr. John Cherry represented the EEP on the Expert Panel for development of the new five-year grant and met September 11-13, 1995, November 14-17, 1995, and January 25-27, 1996.

- Dr. David Hsi represented the EEP during the presentation of the five-year plan to USAID April 23-24, 1996.

US AID Program Manager

The Program Manager, Dr. Terri Hardt, AID/R&D/AGR, interacted with the CRSP on a continuing basis, and provided the primary linkage for the CRSP with AID.

Activities:

- As an ex officio member, participated in the Board and Technical Committee Meetings.

- Provided necessary actions on travel clearances, equipment purchases, etc.

- Coordinated and promoted CRSP Council activities for the Peanut CRSP.

- Provided liaison with the CRSP for all information from AID important to the functioning of program. Particularly, provided information needed for the preparation of the five-year plan.

BIFADEC Liaison

The liaison, Mr. William Fred Johnson, provides a link with BIFADEC for information and actions important to the Peanut CRSP. Since the BIFADEC was dissolved, this role diminished.

Activities:

- Provided information regularly to the CRSP, including participating in the monthly CRSP Council Conference calls.

- Provided periodic counsel to the Management Entity related to CRSP - USAID relationships.

PEANUT CRSP IN RELATION TO EXPORT COMPETITION

Host countries of the Peanut CRSP do not compete in the edible peanut export market. Some West African countries, particularly Senegal, export peanut oil and oil meal, but the United States does not export oil or oil meal. The Senegal oil and meal export is by agreement with France and is not open to other countries on a competitive basis. The U.S. Department of Agriculture, Foreign Agricultural Service data lists minor amounts of peanut export from some host countries, for example, from Mali. This export is probably not export of edible peanut, but oil seed being shipped to a mill in another country in the Region for extraction.

PLANS FOR 1996 and Beyond

Following September 30, 1996 the old grant will be closed out and a new, five-year grant initiated. Scientists participating in the Peanut CRSP have made great progress in developing new technologies for peanut production and processing. There are, however, new and continuing problems facing peanut growers, processors, and consumers: 1) Mycotoxins are still significant health hazards; 2) There is great scope for increasing yields and reducing dependence on chemical pesticides; 3) Peanut products have great, untapped potential for improving human nutrition.

Development of the new grant included:

- Expert panel of eight U.S. and overseas individuals with expertise in international Peanut Research and Development was assembled. The Panel developed the scope and framework for the new grant; developed and reviewed the request for proposals and recommended those to be submitted as full proposals; reviewed full proposals and recommended those to be included in the grant.

- The Expert Panel process resulted in an expanded scope of the technical program and expanded collaboration - U.S. universities from four to ten, world regions from threeto six, and host countries from ten to 14.

- The grant was negotiated with USAID and significant progress was made with the U. S. and Host Country collaborators toward implementatio of the program.

- Information on the new program is available on the Peanut CRSP World Wide Web Site which can be located at http://www.griffin.peachnet.edu/pnutcrsp.html.

- Further information can be obtained from the Peanut CRSP Management Office.

TX/BCP/WA
Disease Resistant Peanut Varieties for Semi-Arid Environments

Texas A&M University
Institut Senegalais de Recherches Agricoles, Senegal
University de Ouagadougou/Institut Development Rurale, Burkina Faso
Institut Economie Rurale, Mali

O.D. Smith, Principal Investigator, TAMU; O. N'Doye, Senegal; P. Sankara, UO-IDR, Burkina Faso; Kodio, O. IER, Mali; Co-Principal Investigators. C.E. Simpson, A.M. Schubert, W.J. Grichar, TAMU, Co-Investigators.

INTRODUCTION

Productive cultivars that will withstand the stresses of environments are effective components of sustainable agriculture and the welfare of man and animals. Seasonal and within season irregularity in rainfall; soils varied in fertility, water holding capacity, and acidity; diseases; arthropods; weeds; and other factors make profitable and stable food production a challenge. This challenge is heightened where economies prevent effective use of soil amendments, pesticides, irrigation, and other yield enhancing production practices. Such is the situation in West Africa where relatively stable production is a must as storage systems do not allow the carryover of food supplies from favorable years to those in which supplies are deficient. Peanut is an important food crop in much of West Africa and in the U.S. The development of cultivars to better meet the growing and changing needs of the world is important.

Rainfall was an imposing factor to production in 1995. Planting was delayed in most of the West African areas addressed through this project because of inadequate moisture. Subsequent intermittent drought caused stress and slowed plant development. However, in areas such as in southwestern Burkina Faso conditions became favorable for heavy foliar disease development which further reduced productivity. In Texas, the season also was varied. Drought and high temperatures caused major plant stress in much of the state; however, conditions changed in northern Texas during August and moisture was more than abundant and diseases damaged production. Consequently, yield and quality were affected. As with the growers, such yearly extremes present a major challenge in the interpretation and utilization of research results.

MAJOR ACCOMPLISHMENTS

"Tamrun 96", formerly identified as Tx896100, a new runner-type peanut adapted for production in the Southwest U.S. peanut production region was released in the late spring of 1996. Seed increases made in 1995 and 1996 brought the total supply to approximately 59 tons which will be used and sold by the Texas A&M University Foundation Seed Services for multiplication and Registered seed production. The principal advantages of the new variety include partial resistance to tomato spotted wilt virus, partial resistance to *Sclerotium rolfsii* and some other soilborne diseases, high yield, and good seed size.

Five runner-type breeding lines with good yields in multiple location trials in Texas and Oklahoma and low relative damage from sclerotinia blight (caused by Sclerotinia minor) in a 1995 disease nursery were selected for pure seed increases of 0.1 to 1.0 acres each. Shelling, sizing, and quality will be evaluated along with 1996 multiple location two-state test data for decisions regarding preparation of proposals for release.

Selections from BC5 interspecific derived hybrids involving rootknot nematode by susceptible parents at a heavily infested site on-farm test showed very positive response for resistance to nematode infection.
Second year data confirmed the repeatability of differences between genetically high versus low oleic acid Spanish F_2 derived populations grown at diverse locations in Texas at altitudes ranging from 370 to 3300 ft. In general, however, the O:L ratios were lower on the Southern High Plains than in South Texas.

Runner-type segregates with improved oil chemistry and sclerotinia blight and/or spotted wilt virus resistance have been identified and employed in a back crossing program.

GOALS

-Develop superior peanut lines and varieties for stable and profitable production in West Africa and in Texas.
-Enhance the quality of peanut products through breeding and management.
-Strengthen and increase the effectiveness of peanut improvement programs through interaction, training, encouragement, and collegial effort.

OBJECTIVES

- Develop, increase, and release peanut varieties with increased stability and improved productivity in important ecological zones of West Africa and southwest U.S.A.
- Incorporate quality oil traits into improved lines with resistance to sclerotinia blight, TSWV, and leaf spot.
- Assess the potential usefulness of various candivars and cultivars in Sahelian West Africa and identify production constraints which must be addressed in the development of successful varieties for the region.
- Enlarge and enhance the germplasm resources of the collaborative peanut improvement programs.
- Develop improved methods of evaluating disease resistance.
- Assess chemical quality, aflatoxin suppression, and arthropod response characteristics in the evaluation of candivars.
- Encourage participants and increase the expertise of host country scientists in the development and culture of improved peanut varieties.

APPROACH

-Field screen and test selected populations and breeding lines derived from short-growth duration and adapted parentage for identification and verification of useful, short growth duration, agronomically desirable genotypes.
-Continue development and selection of populations with early maturity, disease resistance (leaf spot, spotted wilt virus, rosette virus, rust, soil borne diseases) , high quality, and productive parentage for evaluation and further selection in collaborating country programs.
-Interact and collaborate with other CRSP projects, especially those active in West Africa, to increase research effectiveness and gain needed information on quality factors, including aflatoxin production, of elite breeding lines and germplasm, soil characteristics in relation to differential variety performance and/or cultural management, and disease and arthropod resistance.
-Cooperate with ICRISAT, CIRAD-CA, and other regional programs in addressing the important constraints to peanut production in West Africa.
-Evaluate the disease reactions of select germplasm lines and checks in Burkina Faso with relatively high rainfall where disease pressure is conducive to effective evaluation and screening.
-Assist and encourage host country scientists in achieving project goals and developing effective peanut research programs.

ORGANIZATION PERSONNEL

U.S. Lead Institution	Texas A&M University (TAMU)
Principal Investigator	Dr. Olin D. Smith, Department of Soil and Crop Sciences (S & CS), TAMU, College Station (CS)
Co-Investigators	Dr Charles E. Simpson, TAMU Research and Extension Center, Stephenville Dr. A. M. Schubert, TAMU Research and Extension Center, Lubbock Mr. W. J. Grichar, TAMU Research & Extension Center, Yoakum
Cooperators	Dr. Nancy Keller, Department of Plant Pathology & Microbiology. TAMU, CS
Research Associate	Mr. Brent Besler, TAMU Agri. Research Station, Yoakum
Research Assistants	Mr. Michael Baring, Dept. of S & CS, TAMU, CS Mr. Pete Higgins, TAMU Res. and Ext. Ctr., Stephenville Ms. Yolanda López, Dept. of S & CS, TAMU, CS Mr. Justin Tuggle, Dept. of Plant Pathology & Microbiology, TAMU, CS
Budget Analyst	Mr. Robert Koch Owens, TAMU Research Foundation, CS
Secretary	Ms. Celia Tabien, Dept. Of S & CS, TAMU, CS
Institutional	Dr. Ed Price, Assistant Vice Chancellor, TAMU System, CS Dr. E.C.A. Runge, Head, Dept. of S & CS, TAMU, CS

Burkina Faso: University of Ouagadougou

Collaborators:	Dr.Philippe Sankara, ISN/IDR, University of Ouagadougou Dr. Mahama Ouedraogo, University of Ouagadougou
Administrative:	Dr. Idrissa Dicko, Director IDR/ISN, University of Ouagadougou

Senegal: Institut Senegalais de Recherches Agricoles (ISRA)

Collaborator:	Mr. Ousmane Ndoye, ISRA/ CNRA, Bambey
Administrative:	Dr. Dogo Seck, Directeur, ISRA/CNRA, Bambey Dr. Jacques Faye, DG, ISRA, Dakar

Mali: Institut Ecomonique Rurale (IER)

Collaborator:	Mr. Ondié Kodio, Centre Regional de Recherche Agronomique de Same, Kayes
Administrative:	Dr. Dr. Aly Kouriba, Directeur de Centre, Kayes Dr. Omar Niangado, DG, IER, Bamako

RESEARCH ACCOMPLISHMENT

Yield Trials

Yield trials were conducted in each of the collaborating countries. Additionally, for the first time HC collaborators in Senegal, Mali, and Burkina Faso conducted a test with entries in common, called the WASEPT test, with similar data being collected by each. Because of personnel changes, the collaborator in Senegal traveled to Mali to demonstrate the use of U.S. grading equipment. This was our first opportunity to be able to analyze statistically the adaptation and other traits of entries across the region.

WASEPT trials. Fifteen breeding lines plus checks and some local additions were tested in Senegal, Burkina Faso, and Mali under the hospices of the Peanut CRSP. The materials tested consisted of hybrid derived breeding lines produced in Texas from crosses of West African and U. S. varieties and germplasms known to have earliness and adaptation to Senegal. Check varieties included one or more prominent varieties of a similar maturity group for each of the countries. Tamspan 90 was included except in Mali. Each test consisted of four replications of 5-row plots 3 or 6 m in length. Data collected included plant stand 20 days after planting (DAP), days to 50% flowering, leaf spot ratings 45 and 75 DAP, pod, seed, and haulm yield and grade. Planting at Farakoba and Mali were late because of a late start in the rainy season but planting conditions were good and emergence was good, in general, except for Fleur 11. Flowering (50%) of the entries occurred 25 to 27 DAP except in Senegal where 50% flowering occurred 42 to 44 DAP. Low temperatures and cloudiness are suspected for the late flowering in Senegal. Leaf spot scores of over 8 (ICRISAT scale) were recorded for most plots at 75 DAP at Farakoba, and by harvest on Nov 13 almost all plots were defoliated. However, in Senegal disease pressure was very low and heavy sequificient defoliation was not a problem.

Pod yields varied depending on location. In Mali, there was no difference among varieties for pod yield although yields ranged from 434 to 1019 kg/ha. Haulm yields ranged from 1121 to 2229 kg/ha but were not different (P=.05). In Farakoba, pod yields were very low because of low plant population (particularly at harvest), high foliar disease pressure, and a late harvest. Grades were low also with a range of 25 to 52%. Because of the low shelling percentages and grades of some entries, the pod yields did not reflect the actual usefulness of the production as well as TSMK yield. For example, TS 32-1 ranked first in pod yield with a 19% advantage over the entry second in rank but was 11% below the second variety in kg TSMK/ha. Yields were good at Gampela (1120 to 1928 kg/ha) and grades ranged from 56 to 70%. At both Burkina locations local checks ranked 1st for pod yield but not for TSMK yield.

In Bambey, the average pod yield was 760 kg/ha and the highest was for Fleur 11 (1377ka/ha) which exceeded 55-437 by 28%. Pod yields of five of the breeding lines equaled or exceeded checks 55-437, TS 32-1 and 47-10. All haulm yields were good with Tamspan 90 the highest producing 5738 kg/ha of haulm and 1012 of pods. Grades ranged from the mid 50's to low 70's. In Mali, Fleur 11 ranked first in the test but the entries were not different statistically.

Relative yields of the entries varied among tests. Fleur 11 ranked well in Senegal and Mali but was only average in Burkina Faso where emergence was poor. Similar yield differences, but not necessarily related to plant emergence and survival, were apparent for the breeding lines. The CV's of some tests were pretty high which also could add to apparent interactions. Nevertheless, this collaboration in these neighboring host countries provides very useful and mutual benefit by enhancing communication among HC collaborators, learning the adaptation of germplasms, and hopefully can prove useful in identifying useful new cultivars with adaptation to a spectrum of environments as occurs in this region.

Burkina Faso

WAPEP trials. Thirty-three entries including 4 check cultivars were compared in replicated 2-row x 3m plots at four locations: Tenkodogo, Gampela, Bobo Dioulasso, and Niangoloka. Yields ranged from good to poor among locations. Emergence was poor at both Niangoloka and Bobo Dioulasso, and early and late leaf spot,

termites, and heavy weed growth further reduced yields. Skips were replanted in the Bobo test but still emergence was poor and rainfall was inadequate. The plants harvested as a percentage of seed planted averaged 41 and 43%, respectively, at the two sites but varied within tests from below 20% to 60%. The percentage of total sound mature kernels of many entries was low, also. Because of the large variation among entries in %TSMK and yield, the yield of TSMK per unit area was very poor, especially at Niangoloka where then average was 145 kg/ha and some lines had less than 50. Furthermore, the yields and grades did not parallel among the entries so that some lines with average pod yields had very poor yields of good seed. The relative performance of several entries varied at the two southern test locations but some, especially Tx896102, did well at both sites. At Bobo it produced 36% more TSMK than the best check (different at P= .05), while at Niangoloka the different was not significant but 14% over the best check. At Gampela and Tènkodogo the yields were much better with average TSMK/ha yields of 1479 and 490, respectively. The percentage of seed that produced harvested plants was 62 and 56%, respectively. In general, the best performing lines in the southwest were not so good at these locations. Only the breeding line Tx903839 was in the top statistical group for TSMK yield in both tests.

Senegal

Yield tests were continued on selected advanced lines, and dormancy in Spanish peanut, and leaf spot resistance at Nioro. No decision has been made toward release of neither PC79-79 for oil production nor H75-0 for edible use. Leaf spot pressure was not as heavy as in previous years.

Administrative changes in ISRA were made and the duties of Mr. Ndoye were increased to include other leguminous crops. Planting at Bambey was at a minimum because of the late transfer of Mr. Ndoye and his need to adequately familiarize himself with the materials and needs there.

Mali

Tests additional to the multinational test discussed previously included the evaluation of 13 early Spanish lines in the western short season region near Kayes. Mr. Moussa Sanogo, project assistant and principal communicator, has gone for advanced training and a full country report is not available at this time.

Disease Resistance

Leaf spot.

Burkina Faso

Populations of both interspecific and cultivated derived leaf spot resistant materials were planted at Farakoba. The interspecific materials consisted of early generation selected backcross families and parental lines developed through Dr. Ouedraogo's dissertation work at Texas A&M University. The cultivated were F_3 populations from crosses of BNV Nama by 73-33 and Andru 93. "Nama" is a late maturing, short statuted, spreading bunch peanut with small seed that was collected in Burkina Faso. It was identified first in Burkina Faso and later in Texas to withstand heavy leaf spot infection.

Leaf spot was heavy in southwest Burkina Faso and the resistance of none of the interspecific families as a whole was adequate. Plants with reduced infection were selected from both the interspecific and Nama crosses and planted for continued study in 1996.

Texas

Selections were made in 1995 among spaced plantings of 8 interspecific derived $F_{2:5}$ populations selected in the F_2 on the basis of disease reaction and agronomic traits. Yield, pod characteristics, kernel size, kernel weight, and kernel color, and plant disease scores (Florida Scale) were used as selection criteria. Parentage of the selections included adapted varieties, introgression materials from Dr. C. E. Simpson and from ICRISAT. Seed from selected plants were grown in plant rows in 1996 for evaluations. Disease pressure was augmented both years by applying inoculum for early leaf spot between 60 and 70 days after planting both seasons. Evaluations

were made upon occurrence of disease symptoms. Spaced plantings of pure lines were also planted for evaluations using the same protocol. The interspecific progenies ranged in early leaf spot reaction from scores of 2 (few lesions in lower canopy) to 7 (75% defoliated), and in plant yield from 24 g to 215 g of pods. Mean values for Florunner and Southern Runner checks were 98 and 79 g, respectively. Additionally, cycle 0, 1, and 2 parents used in recurrent selection for yield and leaf spot resistance were increased for comparison of progress.

Concern has been expressed that some of the early leaf spot resistance evident in the early interspecific crosses had been lost in subsequent generations of back crossing. Re-examination of BC_2 populations thought to have the higher levels of leaf spot resistance was initiated in 1993 and continued through 1996. Fourteen runner-type plant progenies were selected from 89 BC_2F_5 plant rows grown in 1995, which derived from about 800 plant rows in 1994, for a replicated disease evaluation and yield test in 1996. Disease was augmented the latter two years through the addition of inoculum, and irrigations to enhance disease development. Early leaf spot disease pressure was heavy and the severity of early leaf spot varied markedly among entries in the test. Yield, grade, and fruiting traits of the most resistant are being evaluated. Decision regarding potential advantage to be derived from using any of these lines as parents as opposed to more advanced backcross material will be made following completed data collection and evaluation.

Six advanced lines under increased scrutiny for yield, grade, and sclerotinia blight reaction, yield, grade, and other agronomic traitswere evalutaed for leaf spot resection, yield & grade at Yoakum. At-108 was the most suseptible. TX901639-3 showed considerable early leaf spot resistances and its progenies also showed useful resistance to tomato spotted wilt virus and sclerotinia blight in separate tests.

Populations of BNV Nama x 73-33 and Andru 93 were grown under augmented early leaf spot pressure at Yoakum. Segregation for leaf spot was readily apparent and seed size varied although on the whole they were small. Composites of plants with seed medium to small runner in size were selected. Sub-lots of the composites were screened mechanically with both 16/64 and 18/64 screens for separate plantings prior to planting in 1996. Hopes are that at some point exchange between Burkina Faso and Texas for comparissons with local selection in each locale.

Sclerotinia blight

Crosses were made of high oleic parents and three advanced generation runner-type lines with sclerotinia resistance. Additional backcross 1 and backcross 2 crosses were made of sclerotinia resistant Tamspan 90 and high oleic germ plasm. Single and backcross Spanish and runner-type populations were increased in a winter nursery and field tested at sites with and without sclerotinia were planted in 1996.

Additional screening was made of new and previously selected single, 3-way, and backcross selections . A total of 189 selections plus checks were screened for the first time, while repeat evaluations were made on 218 lines. Disease scores in conjunction with traits as pod size, seed size, pod shape and seed color were used as criteria for continuation in preliminary yield tests.

Six of the advanced generation runner-type breeding lines tested at multiple locations with and without sclerotinia pressure in 1995 were chosen for continued multiple location testing in 1996 in Texas and Oklahoma. The Oklahoma tests were in cooperation with Dr. Hassan Melouk, USDA-ARS, Stillwater. Additionally, pure seed increase was initiated in from plant selections among five of the lines. Field 1996 plantings range in size from 0.1 to 1.2 acres each. Selection was effected among plant derived families for uniformity, and plant and fruiting characteristics. Shelling and quality data are to be collected. Crosses were made of three of the lines with parents having improved oil composition with hopes of combining the disease resistance and improved storage quality. Populations were increased in Puerto Rico and some 400+ plants were selected. These were planted for further increase and observation, and in a sclerotinia disease nursery in Texas in 1996. Ten-seed

samples were also analyzed for oil composition. Disease, agronomic and oil composition will be included in the selection criteria.

Two additional yield tests of about 20 entries each were compared with appropriate checks under enhanced disease conditions at Stephenville. Entries for these tests were selected from the 1994 screening nursery for first time yield comparisons in 1995. Six of those lines were selected for multiple location tests in 1996 and were a part of the Texas and Oklahoma trials in 1996.

Southern Blight

Eleven varieties (4 of which were recent releases) and one breeding line were evaluated for southern blight, yield, and grade using 0,2, and 4-spray fungicide treatments at the Texas Agricultural Research Station near Yoakum, TX. The test, with 4 replicates, was arranged in a split-plots. For the 2-spray treatment, Folicur 3.6F fungicide at 7.2 oz/ac was applied 60 and 88 DAP, and for the 4-spray treatment the same fungicide was applied 60, 74, 88, and 102 DAP. 42 g/plot of supplemental *Sclerotium rolfsii* inoculum on an oat medium was distributed over the row to enhance disease development. Cultivars were dug when mature based on a modified hull scrape test and disease loci were determined immediately following inversion of the plots. Disease loci were defined as $\leq$ 1 ft of consecutive damage in a row.

Disease incidence was heavy. Mean averages for southern blight disease incidence were 9.4, 7.6, and 7.2 hits/40 ft. of row, respectively, for the 0, 2, and 4 spray treatments. Southern Runner with 14.5, 11.0, and 12.0, and Andru 93 with 12.5, 12.0, and 11.0 hits for 0, 2, and 4 spray treatments, respectively, showed the most infection. None of the varieties showed benefit of the 4-spray over the 2-spray treatment. Tamrun 96 exceeded the next highest entry by 649 lbs/ac and did not show benefit from fungicide application.

Nematodes

Analysis of the 6th backcross generation was completed and the 7th backcross was initiated. The number of recurrent parents was increased by three to include some parents with improved oil chemistry. Thirty-seven cross combinations were made for the 7th backcross. Additionally, seed from the 5th backcross was increased sufficiently for on-farm comparisons in 1996. The vine condition of resistant lines was much improved over that of commercial checks suggesting that distinct yield advantages will occur.

Rosette Virus

Thirty-seven lines and three checks were challenged to rosette virus infection at Niangoloka. Rainfall was short of needs, emergence was poor and weed growth became heavy. Rosette was first noted 45 days after planting. Rust and leaf spot was present but less than in 1994. Only plants without symptoms of rosette were harvested.

Spotted Wilt Virus

Virus incidence was determined on 52 Tx896100 plant progenies under disease enhanced conditions in a grower field near Pearsall, Tx. Disease was enhanced in the 3-replicate test by bordering each plot on one side by the susceptible variety Tamrun 88. Disease incidence was high and most of the plants in the susceptible spreader rows died prematurely. Virus readings were taken on four dates during the season by Dr. Mark Black, cooperating Extension Plant Pathologist, at Uvalde, TX. The diseased plant incidence varied among entries but the differences were not significant (P=.05). Nevertheless, we decided to omit three lines from the breeders seed composite because of near significant differences in virus incidence.

Seven lines plus Southern Runner and Florunner were compared in 4-replicate combine harvest size plots. Each plot consisted of 4 rows 70 ft. in length and each plot was bordered on each side by two rows of Tamrun 88. Disease incidence was estimated on four dates by Dr. Mark Black. Four of the lines exceeded Florunner in value per acre by 80% or more with the highest average yield of 4092 lbs/ac produced by Tx896100. Southern Runner averaged 3157 lbs/ac compared to Florunner at 2116.

Sixty-two $F_{2:4}$ families were grown in triplicate in 1995 under augmented SWV pressure in a growers field near Pearsall Texas. SWV incidence was recorded on four dates as in the other tests. The families were comprised of two and three parent combinations of Southern Runner, NC 343, and adapted breeding lines and varieties as an attempt to identify higher levels of SWV resistance than in current use in the program. Following evaluation of disease, yield, grade, seed size, and other data, 21 were retained for further evaluation in 1996. These, plus checks, were planted in a 4-replicate yield test in 1996. Disease severity ratings and agronomic data are being collected and assimilated.

Peanut Quality

Eighteen $F_{2:5}$ spanish families with varied O:L ratios were again compared in South, Southeast and Northwest Texas. The purpose of the tests was to confirm 1994 data on the stability of the O:L ratios in the varied climates; that is that lines with elevated O:L ratios at low (250 ft.) elevations would also show improved oil chemistry at elevations of 2500 ft. The populations derived from crosses of spanish varieties x unadapted, unusually high oleic acid, Spanish-type parents. The results confirmed the 1994 results which indicated marked improvement at all locations among lines with the genetically superior oil chemistry. As in 1994, the O:L ratios were higher among the high O:L lines at the lower elevations than in West Texas. No differences in the rate of emergence between the high and low oleic entries was apparent as has been reported in cotton. As in 1994, the yields of these single cross high oleic lines was inferior to the best commercial Spanish check varieties at all locations.

BC_2 high oleic spanish populations were produced and selections made for the third backcross. Some 84 single and first backcross selections were tested for yield and sclerotinia reaction in Texas and Oklahoma.

<u>Other studies</u>

Germplasm Characterization

Characterization of the Malian peanut collection was continued at the Kita Station. Yield, seed size. Groth duration in trems of flowering and mature pods, shelling percentage, and disease reaction were evaluated with checks of varied maturity groups.

Earliness Study

Some 250 to 750 plants each of 20 F_2 and F_4 populations from crosses of very early maturing spanish x commercially acceptable runner populations were grown in spaced plantings at Yoakum. From this number, 189 plants were selected based on apparent earliness and reasonable runner agronomic type for continued study in 1996. Pod examinations during early fruiting revealed notable differences among the selections and checks in rate of maturation. Selected rows were harvested for continued study of both the germplasm and the evaluation technique.

TRAINING

Name	Sex	Univ.	Degree	Date	Support
Lopéz, Yolanda	F	TAMU	PhD	Aug 1998	Partial
Tuggle, Justin	M	TAMU	PhD	May 1997	Partial

PRESENTATIONS AND CONFERENCE PARTICIPATION

Smith, O.D., M. C. Black, and M. R. Baring. 1996 Breeding tomato spotted wilt virus resistant peanut varieties for South Texas. Twenty-eighth Annual Meeting of APRES, Orlando FL. July, 1996

Tuggle, J. C., O. D. Smith, J. L. Starr, and B. A. Besler. Selection for early leaf spot resistance and high yield within interspeicfic breeding lines of peanut. Twenty-eighth Annual Meeting of APRES, Orlando FL. July, 1996

Simpson, C.E. Pollen storage for cross pollination in Arachis. Twenty- eighth Annual Meeting of APRES, Orlando, FL, July, 1996

Ouedraogo, M., P. Sankara, and O. D. Smith. 1996. Performance of some selected peanut lines in West Africa. ICRISAT West African Regional Workshop. Accra, Ghana.

Besler, B.A., W.J. Grichar, and O.D. Smith. 1995 Germination of selected peanut varieties in small grain residue extracts. Twenty-seventh Annual Meeting of APRES. Charlotte, NC.

PUBLICATIONS

Besler, B.A., W.J. Grichar, and O. D. Smith. Susceptibility of Peanut Varieties and breeding lines to southern stem rot. Peanut Sci. (Accepted).

Goldman, J.J., O. D. Smith, C. E. Simpson, and H. A. Melouk. Progress in breeding sclerotinia blight resistant runner-type peanut. Peanut Sci. 22:109:113. 1995.

Ouedraogo, M., O.D. Smith, C.E. Simpson, and B.A. Besler. 1995. Combining ability for components of resistance to early leaf spot and yield of inter- and intra specific peanut lines. OIL 2:149-152.

Smith, O.D. and C.E. Simpson. 1995. Selection of peanut varieties. *In* Peanut Health Management. APS Press.

Plans for 1996

Field plantings were restricted largely to those for which local finances could complete in 1996-97.

NCS/BCP/TP
Peanut Varietal Improvement for Thailand and the Philippines

North Carolina State University
University of the Philippines at Los Baños

Thomas G. Isleib, Principal Investigator, NCSU
Remedios M. Abilay, Principal Investigator, UPLB, Philippines

INTRODUCTION

Improved peanut cultivars are essential in an integrated plan to augment yields and profitability of peanut for farmers in Thailand, the Philippines and North Carolina. Crop environments and cultural practices used in peanut production vary greatly among and within peanut-producing areas. In Thailand, peanuts can be produced during a brief period of the rainy season before rice planting, after rice harvest using residual soil moisture, or in upland field conditions during the rice-producing rainy season. Irrigation may also allow some farmers to produce peanuts during the dry season. Similar conditions are present in the Philippines where the duration of the rainy season can vary between three and six months over areas where peanuts are grown. The most productive growing season in the Philippines continues to be after rice, but breeding efforts are needed for peanut to fit in other cropping systems including the shaded floors of coconut groves and upland areas with acid soils.

Constraints to production vary from place to place and system to system. In almost any situation, foliar fungal pathogens, soil-borne pathogens, and arthropod pests would destroy the peanut crop if uncontrolled. The highly capital-, chemical-, and energy-intensive production system used to achieve control in the United States is not practicable nor desirable for growers in Thailand or the Philippines where integrated programs of disease and pest management must be used. The prevailing regulatory philosophy in the United States emphasizes conservation, food safety, and environmental protection, leading to reduced use of pesticides, leading to more emphasis on the integrated approach. Host plant resistance to economically important pests must be a salient feature of this approach.

ACCOMPLISHMENTS

Philippines

Philippine seedboard variety PSB Pn 1, tested as UPL Pn 10, released in 1992 and marketed as 'Biyaya 10', was multiplied and distributed to growers in the state of Isabela where it was well accepted by local growers. Biyaya 10 yielded significantly more peanuts than BPI Pn 9 (2.44 t ha^{-1} vs. 1.95 t ha^{-1}) in pilot tests.

Three new entries were included in the National Cooperative Test conducted at nine locations across the Philippines in May 1996. In trials perfomed at IPB, all three have had greater yields, larger seeds, and improved insect resistance compared with Biyaya 10.

Thailand

Thailand's participation in the project was terminated in FY1994-95 following Thailand's graduation from USAID client status.

United States

Cylindrocladium black rot-resistant breeding line N90013E was released in 1996. Registered seed was produced in the 1996 growing season; certified seed will be produced in 1997 and the cultivar will be in the hands of peanut growers in 1998.

Germplasm release was planned for several genotypes derived from interspecific crosses and possessing resistance to early leafspot (Cercospora arachidicola), root-knot nematodes (Meloidogyne incognita), or insects (Helicoverpa zea, Empoasca fabae, and Diabrotica undecimpunctata).

Numerous transgenic lines with the coat protein (CP) gene from Peanut Stripe Virus (PStV) have been recovered. Efficacy testing has identified several lines with greatly enhanced resistance against the virus. Transgenics are fertile, and the trait is transmitted to self progeny.

GOALS

To increase yields and quality of peanut in the U.S. and cooperating Asian countries while decreasing the dependency on chemical inputs. This should improve not only the economic situation for farmers but also have a positive impact on the ecology and sustainability of agriculture. Breeding efforts need to be directed toward development of cultivars that fit current and future cropping systems and marketing potential.

OBJECTIVES

North Carolina

1. Aid in the identification of useful genotypes and implementation of material in crossing programs for use in the Philippines and Thailand.

2. Obtain greater understanding of the interrelationships between peanut host and pathogen or environmental stress that occurs in peanut-growing areas of the Philippines, Thailand, and the United States.

3. Improve breeding and selection techniques. Break barriers prohibiting incorporation of desired traits into adapted material via interspecific crossing and molecular engineering techniques. Improve screening techniques for resistance to pests and tolerance to stress.

Thailand

1. To develop peanut cultivars that can be established in specific cropping systems and seasons. Cultivars with high yields, early maturity, large seeds, resistance to rust, leafspots, Aspergillus flavus, Aspergillus crown rot, Sclerotinia stem rot, TSWV, stripe virus, and insects and drought tolerance would be incorporated for specific growing seasons and cropping environments.

2. Provide information on plant pathology required for development and utilization of disease-resistant cultivars.

3. Develop cultivars for the boiling-type market.

Philippines

1. To develop stable peanut cultivars that are high yielding, resistant to major foliar diseases (rust and late leafspot [Cercospora personatum]), leafhopper damage, longer seed viability for specific growing environments - upland optimum growing conditions, partial shaded and highly acidic soils.

2. To identify peanut genotypes tolerant to intercropping under coconut and on highly acidic soils.

ORGANIZATION AND PERSONNEL

A.	U.S. Lead Institution:	North Carolina State University, Raleigh
	Principal Investigators:	Dr. Thomas G. Isleib, Dept. of Crop Science
	Co-Principal Investigators:	Dr. Marvin K. Beute, Dept. of Plant Pathology
		Dr. H. Thomas Stalker, Dept. of Crop Science
		Dr. Arthur K. Weissinger, Dept. of Crop Science
	Research Associates:	Dr. Barbara Shew, Dept. of Crop Science
	Technicians:	Ms. Joyce Hollowell, Dept. of Plant Pathology
		Ms. Susan Copeland, Dept. of Crop Science
		Mr. Donald Parry, Dept. of Crop Science
		Ms. Gerry Phillips, Dept. of Crop Science
		Mr. Philip Rice, Dept. of Crop Science
	Institutional Representative:	Dr. David A. Knauft, Head, Dept. of Crop Science
B.	S.E. Asian Counterpart Institutions:	University of the Philippines at Los Baños (UPLB), Philippines
	Coordinator (Philippines):	Ester L. Lopez, PCARRD, Philippines
	Principal Investigator (Philippines):	Dr. Remedios M. Abilay, UPLB, Philippines
	Co-Investigators (Philippines):	Ms. Rosie Aquino (Ilagan Exp. Sta., Isabela)
		Dr. Marina P. Natural (UPLB)
		Dr. Henry P. Samonte (UPLB)
		Dr. Vermando C. Aquino (UPLB)
		Mr. Apolonio M. Ocampo (UPLB)
		Mr. Edwin del Rosario (UPLB)
	Research assistants:	Ms. Leonila A. Lantican
	Cooperating Agencies in the Philippines:	Ilagan Experiment Station (IES)
		Bicol Experiment Station (BEST)
		Quezon Agricultural Experiment Station (QAES)
		Philippine Coconut Authority (PCA) at Bago Oshiro, Davao City
		Mayors Office and Department of Agriculture at Guagua, Pampanga
		Aurora Integrated Area Development Project (AIADP)
		University of Southern Mindanao (USM)
C.	USAID Project Officers:	Mr. Robert Resseguie,USAID, Manila
		Mr. Douglas Clark, USAID, Bangkok

APPROACH

Peanut germplasm was introduced to Thailand and the Philippines. Observations on agronomic potential, disease and insect resistance, maturity, drought tolerance and other agronomic traits of the introduced germplasm are made in unreplicated nurseries. Selected lines are grown in preliminary replicated tests to identify lines for further testing at multiple locations within each country and then to on-farm trials. 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 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 evaluated in both countries for potential use in cropping systems peculiar to Southeast Asia such as acid upland soils, shaded floors of tree-crop

plantations, or growth in paddies on residual moisture following harvest of a preceding rice crop. Promising breeding lines are 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 exploiting the natural genetic variation within the cultivated species in the US and the Host Countries, novel genetic information is brought into A. hypogaea in two ways: interspecific hybridization to introgress desirable characters from the wild species, and genetic transformation using microprojectile bombardment or Agrobacterium tumefaciens. In utilizing the wild diploid species, barriers of ploidy are broken by chromosome doubling either before or after hybridization, then hybrids are backcrossed to cultivated parents. Transformation is being used to achieve resistance to peanut stripe virus by incorporating genes encoding viral capsid protein genes.

Short visits to both Thailand and the Philippines have been 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.

RESEARCH ACCOMPLISHMENTS IN DETAIL

I. U.S.

A. Breeding

1. Select intensively for large-seeded adapted lines with resistance to Cylindrocladium black rot (CBR) caused by Cylindrocladium parasiticum.

a. Six lines with superior agronomic performance and resistance to CBR were crossed in a diallel mating along with four agronomically lines exhibiting resistance to early leaf spot. F_1 seeds were sent to the winter nursery at the USDA Tropical Agriculture Research Station at Mayaguez, Puerto Rico. F_2 populations were planted on infested soil at a t site in Bertie Co., NC, for selection of resistant plants.

b. F_2 populations from a partial factorial mating of three sources of resistance to CBR with four breeding lines possessing superior seed attributes and high yield were screened for resistance in a naturally infested field in Bertie County, NC, in 1995. Selected $F_{2:3}$ families were sent for increase to the winter nursery. $F_{2:4}$ families were planted in the infested area in 1996.

c. Selection for resistance and pod attributes was practiced on infested soil among and within $F_{2:4}$ families derived from 68 F_2 plants selected during the 1994 growing season. $F_{4:5}$ progenies were grown in a replicated test on infested soil in 1996.

d. 96 $F_{4:5}$ families and checks were evaluated on infested soil for CBR incidence in a completely random design with unequal replication to allow for variable seed number. These families were increased on uninfested soil at the NCDA Peanut Belt Research Station at Lewiston, NC, and resistant families were screened for pod and seed characters.

e. 57 F_4- and F_6-derived families selected were compared on infested soil with checks for CBR resistance in a replicated test and for yield and market grade at Lewiston in 1995. Four of the lines were first evaluated in the CBR Advanced Test in 1992; three in 1993 and seven in 1994. Five were included in the Advanced Yield Test leading to cultivar release. Also included in the test were six lines derived from a cross between CBR-susceptible cultivars NC 7 and NC 9. These six were found to exhibit partial resistance to CBR.

2. Selection for resistance to early leafspot (C. arachidicola).
   a. 17 F_4- and F_5-derived families from a program of selection for resistance to early leafspot were evaluated for defoliation, yield, and market grades in replicated tests at Lewiston in the 1995 growing season.

   b. 86 F_2 populations derived from crosses between NCSU selections with resistance to early leaf spot and University of Florida selections resistant to leat leaf spot (Cercosporidium personatum) were grown without chemical control of leafspot to permit selection for resistance within populations.

   c. 85 $F_{3:4}$ families derived from 69 leafspot-resistant F_2 plants (selected on the basis of defoliation) were grown without chemical control of leafspot to permit selection for resistance among and within families. $F_{4:5}$ families derived from 40 selected F_4 plants were planted without chemical leafspot control in 1996 for selection of individual resistant F_5 plants with satisfactory pod and seed charactersitics.

   d. 184 $F_{4:5}$ families derived from at least one leaf spot-resistant parent but not previously subjected to the disease were grown without chemical control of leafspot in 1995 to permit selection for resistance among families. Twenty-one selected families were planted in the 1996 Leafspot Advanced Test to obtain estimates of yield potential and grade with and without chemical leafspot control.

   e. 61 $F_{1:5}$ families developed by single-seed descent from crosses involving at least one leafspot-resistant parent were grown without chemical control of leafspot in 1995 to permit selection for resistance within families. $F_{5:6}$ families derived from 30 selected F_5 plants were planted in a replicated test without chemical leafspot control in 1996.

   f. In 1994, three lines selected for resistance to early leafspot and two susceptible lines with excellent yield and grade characteristics were mated with two highly resistant tetraploid lines developed by H.T. Stalker through introgression of germplasm from A. cardenasii into A. hypogaea. F_1 plants were backcrossed to their respective large-seeded virginia parents. BC_1F_1 plants were planted in the 1996 season.

3. Selection of early-maturing large-seeded virginia lines with good storability.
   a. Populations have been developed from crosses of five virginia cultivars with F435, a spanish line with an extremely high ratio of oleic to linoleic acid in the fatty acid profile of the oil. F_4-derived lines homozygous for the two recessive genes conferring the trait were backcrossed to their respective adapted parents. F_1 hybrids were planted in the greenhouse for backcrossing to the cultivar.

   b. 18 advanced F_5- and F_6-derived selections from crosses of NC 7 with NC 9 and Florigiant were evaluated for performance at early (September 15) and conventional (October 1-7) digging dates in 1995. Eleven were retained for further evaluation in 1996. Breeding line N91026E performed sufficiently well in the NCSU-VPI cooperative Early Maturity Test and Peanut Variety and Quality Evaluation (PVQE) program's small plot test that in 1996 it was entered in the PVQE large plot test, the final testing phase before a decision can be made to release a line as a cultivar.

4. Incorporation of arthropod pest resistance into A. hypogaea. 36 $F_{4:5}$ progenies were grown in a replicated test without applications of insecticides or miticides. The lines were selected from crosses between large-seeded lines and a set of arthropod-resistant selections including insect-

resistant accessions GP-NC 343 and NC Ac's 00301 and 00302. These selections were compared with 28 other lines selected from the leafspot-resistance breeding program and descended from GP-NC 343. 12 of the "new" lines and 15 "old" lines were retained for further testing in 1996.

5. Recurrent selection for productivity in a population derived from elite germplasm.98 $S_{2:4}$ families from selected crosses of the sixth cycle (C_6) of recurrent selection were grown for seed increase 1995 growing season at Lewiston. Replicated tests of the $C_6S_{2:5}$ families were planted at two locations in 1996.

6. Recurrent selection for productivity in a population derived from an interspecific cross (A. hypogaea / A. cardenasii). The ten highest yielding selections from the 1994 trial of $S_{0:1}$ families from the fifth cycle (C_5) of recurrent selection were intermated in a half diallel cross. S_0 seeds were sent to Puerto Rico for increase, and the $S_{0:1}$ families were planted in replicated trials at two sites in 1996.

B. Plant Pathology

1. Microplots were infested with known inoculum densities of C. parasiticum or Sclerotinia minor and planted in the fall with wheat, oat, rye, canola, or no cover crop. Peanuts were planted the following spring and disease severity and pathogen survival were monitored. All small grain cover crops suppressed CBR compared with canola or fallow soil, with oat the most suppressive crop tested. Inoculum densities of C. parasiticum corresponded with disease incidence. Although tillage did not affect CBR in general, cover crop effects tended to be more pronounced in tilled plots compared to no-till plots. In contrast, cover crops did not affect incidence of Sclerotinia blight. Incidence of Sclerotinia blight was lower in no-till compared to tilled plots regardless of cover crop.

2. Field tests were conducted to determine the effects of various genotypes, mechanical pruning, and fluazinam applications on Sclerotinia blight progress. Compared to the control (no pruning or fungicide), all treatments were effective in reducing disease on all genotypes in a field with low disease incidence. At a site with high disease incidence, effects of pruning and fungicides varied with genotypes; regardless of treatment, lowest yields were obtained from the susceptible NC 7.

3. Cylindrocladium black rot: The role of root-growth dynamics in development of CBR was examined in field and greenhouse studies. Resistant genotypes produced less root length early in the growth cycle, when the fungus is most active, thereby avoiding contact with the pathogen and infection. Artificial wounding studies showed that some resistant genotypes are efficient at regrowing roots after wounding, which may allow recovery from Cylindrocladium infection. Highly resistant genotypes did not regenerate roots efficiently; this may partially explain why these genotypes yield poorly even though they have high levels of disease resistance.

   We attempted to establish foci of C. parasiticum in a noninfested field of NC 7 peanut. Small amounts of infested soil (35 cm^3) were placed at five sites in each of 24, 3-row plots. Locations of all diseased plants were recorded during the growing season. In the first year of the study, 9% of the foci yielded diseased plants and detectable spread was found from 7% of the foci. Maximum spread was 46 cm. Plots were reestablished the following season. By harvest of the second season, C. parasiticum was detected in 79% of the plots. In four of the plots, more than 95% of one row was diseased. These results demonstrate that even low rates of pathogen establishment, such as could be found with seed transmission, have the potential to cause widespread infestations of C. parasiticum.

4. Cercospora leafspot research. Cooperative efforts with NCSU breeders T.G. Isleib and H.T. Stalker continue to develop germplasm and high-yielding lines of virginia-type peanut with

improved levels of resistance to leafspots. Advanced-generation breeding lines were planted in field isolation plots and evaluated for resistance to leafspots. Incidence of early leafspot, late leafspot, and percentage defoliation were determined and areas under the disease progress curves (AUC's) were calculated. All but two of the breeding lines evaluated had smaller AUC's for early leafspot than current cultivars and four had AUC's that were not significantly different from highly resistant wild species-derived germplasm. Levels of defoliation also were low on these lines.

5. Sclerotium stem rot and Sclerotinia blight: Greenhouse experiments indicated that some wild species of Arachis have high levels of resistance to Sclerotium rolfsii, as measured by lesion development. Field microplots were planted with wild Arachis species and infested with S. rolfsii. Lesion numbers were counted and disease reactions were rated. Several of the wild species had significantly fewer lesions per plant than the susceptible cultivar, but none had higher levels of resistance than the cultivated germplasm NC 3033, as measured by lesion number or disease reaction.

C. Molecular biology. During the current reporting period, we have completed testing of transgenic peanut lines with enhanced resistance to peanut stripe virus (PStV). Several lines from both cv. Toalson and cv. NC 7 have been recovered which carry the PStV coat protein transgene.

Two resistance phenotypes were found. In the first, called “recovery” types, plants show symptoms of systemic infection soon after inoculation. After a period of time (usually one to a few weeks) these plants lose the disease characteristics in newly-emerging tissues. They are subsequently fully resistant to the disease, even upon repeated inoculation.

Another group of transformants shows a fully resistant phenotype. No symptoms are visible either at the time of the original inoculation, or upon repeated inoculation.

All of these lines have now set pods, and some R_1 progeny have been recovered. Molecular characterization continues to determine transgene copy number, transcription level, CP RNA (transcript) accumulation, and CP protein levels. This advanced characterization is being carried out on both primary transformants and first-generation self progeny.

D. Cytogenetics

1. Evaluating germplasm for disease and insect resistance

a. Advanced generation interspecific hybrid lines with A. cardenasii in the pedigree were evaluated in the field for C. arachidicola resistance. Of the 25 lines evaluated, five were selected as having both very high levels of resistance and favorable agronomic traits. Comparable levels of C. arachidicola resistance has not been observed in other 40-chromosome hybrid progenies nor in the cultivated germplasm collection. Although the pod and seed size has been improved, yields still need to be increased before the lines will be agronomically acceptable. The resistance in these lines appears to be stable over years. Eleven of the lines were also evaluated in a spray vs. non-spray test, and leafspot pressure does not appear to adversely affect yields. Attempts to evaluate the lines for late leafspot (C. personatum) in a greenhouse experiment was unsuccessful because of poor inoculation. The experiment is being repeated during the fall of 1996.

b. 214 backcross progenies between the most resistant C. arachidicola-resistant selections and the large-seeded cultivars NC 6, NC 7 and NC 9 were evaluated in the field to confirm resistance levels in selected hybrids. These F_3 backcross progenies were derived from 55 original crosses . Eighty six lines had a leafspot resistance score of 2 or 3 (1 = no disease and

9 = dead), whereas the highly resistant A. hypogaea line PI 109839 had a rating of 4 and several checks were dead at the time of evaluation. Additional selections were made on the basis of high levels of resistance, apparent yield, and good pod shape.

c. Interspecific hybrids derived from A. cardenasii were evaluated in the field for resistance to thrips (Frankliniella fusca), corn earworm (Helicoverpa zea), potato leafhopper (Empoasca fabae) and southern corn rootworm (Diabrotica undecimpunctata) in replicated field experiments. Although no stable resistance to thrips was found, high levels of resistance to corn earworm, potato leafhopper, and southern corn rootworm were confirmed. Seed was increased for future distribution and morphological data was collected to describe the lines in preparation for germplasm release.

d. To improve agronomic characteristics of insect-resistant lines, eighty nine selections from backcross progeny lines with A. cardenasii in the pedigree were evaluated in the field for resistance to corn earworm, potato leafhopper and southern corn rootworm. Fifteen of the lines appear to have high levels of multiple resistances. These 15 lines will be re-evaluated in 1996 for insect resistances and agronomic characters.

2. Introgressing germplasm from wild Arachis species to A. hypogaea

a. Interspecific hybrids between two A. hypogaea cultivars and the species A. cardenasii and A. batizocoi were analyzed for wild species DNA retention in triploids; first, second and third generation hexaploids; pentaploids derived from backcrossing the three generations of hexaploids with A. hypogaea; and selfed progenies from the pentaploids. Chromosome numbers were determined for all plants in all generations of plant materials. Twenty-five randomly amplified polymorphic DNA (RAPD) primers specific for A. cardenasii and 24 specific for A. batizocoi were used in this study with know map locations. Wild species germplasm retention decreased with every generation of selfing after backcrossing, probably due to wild species preferential chromosome loss in the hybrids. This effect was more pronounced in cv. Argentine and in A batizocoi crosses. A decay in wild species germplasm retention was also observed for every cycle of selfing hexaploids, although the reason for this was not clear. Reciprocal recombination and/or translocations are the predominant mechanisms of introgression and no evidence was found for preferential introgression of specific linkage groups. The implications for peanut interspecific breeding is that backcrosses should be made at an early generation, and maintaining interspecific hybrids at a high ploidy level (as currently practiced in the breeding program) is counter-productive.

b. 107 hexaploid and 30 pentaploid lines in advanced generations were propagated in field for seed increases. In addition, hexaploids and pentaploids from the introgression study with A. batizocoi and A. cardenasii were propagated for seed increases. Hexaploids were hybridized with NC 6 and Argentine to obtain additional pentaploid progenies.

c. Two dominant genes conditioning resistance to the root-knot nematode Meloidogyne arenaria were identified in a segregating F_2 population derived from the cross of 4x (A. hypogaea x A. cardenasii)-GA 6a and PI 261942. One RAPD marker was linked to both the gene conditioning galling and to another gene conditioning egg number. The marker was mapped to linkage group 1 on the RAPD map. More than 600 additional RAPD primers were used to find flanking markers, but none proved useful for locating the gene. However, in a parallel study by cooperators, another primer was identified using different molecular marker technologies. Molecular markers should be useful for selecting nematode resistance in future breeding programs.

II. Philippines

A. Germplasm screening for disease and insect resistance. During the 1995 wet cropping system, 129 accessions were evaluated for disease and insect resistance. Three accessions (ICGV 88403, ICGV 88938, and ICGV 86707) were identified as resistant to late leafspot disease. NC Ac 17090 was confirmed resistant to Sclerotium wilt. Two accessions (ICGV 86377 and ICGV 86402) were resistant to leafhopper damage.

B. Breeding for high yield and large seededness. Fourteen individual plant selections were obtained at the F_5 generation from the cross between PSB Pn1 and UPL Pn2.

C. Breeding for shade tolerance. Five hundred one plant selections were isolated from 16 F_5 populations. Selections were based on pod number per plant, seed size, and disease resistance.

D. Breeding for resistance to foliar diseases. Four F_4 populations for rust and late leafspot resistance were further evaluated under inoculated conditions. However, no selection was made due to low disease pressure.

E. Improving disease resistance of PSB Pn1 to rust and late leafspot. Backcrossing of PSB Pn1 with PI 298115 (a source of resistance to rust and late leafspot) resulted in the selection of eight promising backcrosses. The eight backcrosses at the third generation had higher yields than PSB Pn1 and had improved resistance to rust and late leafspot. IPB Pn 88-10-60 BC3 had significantly higher yields than PSB Pn1 and had moderate resistance to rust and late leafspot. Another line, IPB Pn 88-10-50 BC3 (100 seed weight of 56 g) had significantly larger seeds than PSB Pn1 (100 seed weight of 49 g) but moderately susceptible to rust and late leafspot.

F. Selection and inclusion of three entries in the National Cooperative Test (NCT). Three new entries were included in the NCT across nine locations in the Philippines in May 1996. These include IPB Pn88 39-70, IPB Pn88 41-59, and IPB Pn88 36-10. They have higher yields, larger seeds, and improved resistance to leafhopper than PSB Pn1.

G. Studies on yield loss due to late leafspot disease. Field experiments were conducted during 1994 and 1995 to determine effects of late leafspot on yield of three peanut cultivars--namely, UPL Pn 6, UPL Pn 8 and UPL Pn 10. Treatments include leafspot inoculation and fungicide application. Pod and seed weight and number of immature and mature pods were measured. Reduction of pod weight due to late leafspot was highest in UPL Pn 10. Late leafspot reduced the pod yield of UPL Pn 10 by 37% in 1994 and 49% in 1995. Reductions in yield for UPL Pn 8 for 1994 and 1995 were 27 and 28%, respectively. For UPL Pn 6, yield reductions were 21% (1994) and 31% (1995). A corresponding decrease in seed weight was observed for all three cultivars due to increase in the number of immature pods in unsprayed plots.

H. Study on peanut seed viability. A seed viability study using 19 peanut genotypes composed of six recommended varieties and 13 advanced breeding lines was initiated in March 1996. These materials are being evaluated for seed viability under two types of storage--namely, with dehydrant, calcium chloride and without dehydrant; and using pods and seeds.

After 2 months of storage, mean seed germination remained very high under all storage treatments. Genotypic variation was not evident under specific storage procedures but was significant when all storage conditions were averaged. All recommended varieties and most of the breeding lines showed very high seed viability.

Successive sampling of 4, 6, and 8 months of storage will be conducted to verify the effects of the storage variable under study. Effectivity of the $CaCl_2$ treatment as well as the types of materials for storage will be evaluated. The best performers in terms of seed viability after prolonged storage will be identified. Promising lines will be selected as possible sources of desirable seed storability character.

I. Technology transfer

a. Seed production and distribution of improved peanut variety. PSB Pn 1 breeder seed production was initiated in the 1994 wet season in collaboration with the Department of Agriculture at Ilagan Experimental Station, Ilagan, Isabela, involving 55 trained peanut seed growers.

1) Five hundred kg of good seeds were produced during the season. In the 1994-95 dry season, 1500 kg of breeder seeds of PSB Pn 1 were produced. PSB Pn 1 yielded 1.9 t ha^{-1}, while PBI P 9 yielded 1.4 t ha^{-1}.

2) There was a marked increase in the hectarage grown to PSB Pn 1 in San Mariano, Isabela, from 3 ha in 1993-94, 13 ha in 1994-95, to 84 ha in 1995-96. The area grown to BPI P 9 remained relatively constant, averaging 13 ha during the three dry cropping seasons.

3) During the 2-year monitoring period, a total of 4.25 t of improved varieties were distributed. In the 1993-94 dry season, a total of 2.81 t of improved varieties were distributed by the Department of Agriculture at San Mariano, Isabela--0.5 t PSB Pn 1 and 2.3 t BPI Pn 9; and in 1994-95 dry season, a total of 1.6 t of PSB Pn 1 and 2.65 t of BPI Pn 9 were distributed.

4) PSB Pn 1 yielded 25% higher than the traditional variety and 19% higher than BPI Pn 9 in its pilot production in Isabela. In a sample of 27 farmer cooperators during the pilot stage, the pod yield of PSB Pn 1 was 2.44 t/ha compared with BPI Pn 9 with 1.95 t/ha. This pilot testing demonstrated to farmers the advantage of planting improved varieties which gave them higher yield than the traditional varieties they had been using.

5) During the 1995-96 dry season, 85 ha of PSB Pn 1 were grown commercially, while only 13 ha were planted to BPI Pn 9. Hence, this indicates that PSB Pn 1 is becoming more acceptable to peanut farmers in San Mariano, Isabela.

b. On-farm and technology adaptation trials. PSB Pn 1 and PSB Pn 2, released in 1992 and 1994, respectively, were evaluated in on-farm and technology adaptation trials in Villa Miguel, San Mariano and Ilagan, Isabela, in collaboration with the Ilagan Experiment Station. These varieties will be seed-increased further in garden-type seed production in the 1996 wet season.

PSB Pn 1 yielded consistently high for three seasons. It was the preferred variety of seed growers because of its large oblong seeds and easy-to-shell pods.

TRAINING

Surname	Sex	Univ.	Department	Degree	Date degree received	CRSP support
Garcia (Argentina)	M	NCSU	Crop Science	PhD	August, 1995	Total
Menon (India)	F	NCSU	Crop Science	PhD	--	Total
Utomo (Indonesia)	M	NCSU	Crop Science	PhD	May, 1996	Partial

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RELEASES

Release of CBR-resistant NCSU breeding line N90013E as ‘NC 12C’.

PLANS FOR 1996-97

This is a terminal report for the project. No further activity is planned for Thailand and the Philippines. Activities initiated in the US under auspices of this Peanut CRSP grant will be continued usiong other sources of funding if available.

TX/MM/S,G

Mycotoxin Management in Peanut by Prevention of Contamination and Monitoring

Texas A&M University, College Station, Texas
Institut Senegalais de Recherches Agricoles, Bambey, Senegal
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Princicpal Investigators:
TAMU: Marian N. Beremand, Nancy P. Keller, Timothy P. Phillips
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INTRODUCTION

Mycotoxin contamination of peanut poses a serious health hazard to consumers of peanut products throughout the world. Effective mycotoxin management in peanut depends upon the implementation of multiple strategies to prevent contamination or reduce levels of aflatoxins, and other mycotoxins, which may be present in peanut and peanut derived products. This project is designed to conduct experiments which can further identify the mycotoxins and mycotoxigenic fungi present on peanut; define compounds from peanut and/or other organisms that prevent mycotoxin accumulation; implement molecular biological tools to understand the biosynthesis of mycotoxins at the gene level; select peanut germplasm with resistance to aflatoxin production; improve agronomic production practices which reduce the levels of contamination within the field; improve storage practices which minimize further contamination; develop rapid, practical and economical monitoring procedures for the detection of mycotoxins; and devise cleaning, sorting, and detoxifying protocols for removal or deactivation of mycotoxins within peanut and/or peanut derived products.

IMPACTS

Host Countries - Reducing the levels of aflatoxin/mycotoxins in peanuts will help improve the health of the local population, and improve the quality of peanut products. New methods of aflatoxin detection should aid in quantifying levels of aflatoxin present, and aid in justifying the diversion of contaminated peanut into processing for clean-up and/or detoxification. Development of improved marketing procedures, those which provide an incentive for producing aflatoxin-free peanut, will help encourage implementation of aflatoxin preventive measures and removal of mold-damaged seed previous to processing. Newly developed aflatoxin sorption methodologies will allow local villagers and industry to treat peanut oil to reduce aflatoxin levels to a safe level for consumption. Continued screening of peanut genotypes for resistance to penetration by the mycotoxin producing fungi will further help reduce the degree of contamination of peanut at the village level. Identification of local plants which exhibit fungistatic properties or inhibit mycotoxin production can be used in biocontrol programs. Training efforts will improve technology transfer to host countries and be instrumental in the much needed development of molecular biology programs at universities and research institutes. Finally, successful research in the above areas will be applicable to other crops affected by mycotoxins.

In the United States - Research results will lessen the impact of the mycotoxin problem to the peanut industry in the U.S. Molecular and genetic analyses of *Aspergillus* and *Fusarium* mycotoxin genes offer new approaches to preventing mycotoxin contamination and for selecting or producing mycotoxin resistant peanut cultivars. Identification of 'anti-*Aspergillus*/anti-aflatoxin' genes could lead to transgenic peanut cultivars. Newly developed aflatoxin resistant peanut cultivars, adapted to the peanut growing regions of the United States, could greatly reduce the number of Segregation III peanut marketed within the country. Marketing of peanut seed with reduced levels of mold and mycotoxin damage will reduce the costs of processing peanut products. Improved mycotoxin detection techniques should increase the speed and accuracy of analyses, and reduce the costs related to the diversion of contaminated peanut lots. Newly discovered sorption techniques to bind and inactivate aflatoxins, and other mycotoxins should increase the margin of safety to insure that these products are safe for use as food or feed components.

MAJOR ACCOMPLISHMENTS

GHANA

A nation-wide study was conducted to determine the extent of contamination of stored peanut by fungi, especially *Aspergillus* spp. and the aflatoxin levels associated with such kernels. The effects of certain plant extracts on aflatoxin synthesis was also studied using a novel microbial assay involving the norsolorinic acid (NOR) mutant of *Aspergillus parasiticus* Speare. A preliminary report of the study entitled "High incidence of aflatoxins in stored groundnut in Ghana and the effects of some plant extracts on growth of *Aspergillus parasiticus* and/or aflatoxin synthesis" has been published (Peanut Collaborative Research Support Program, Quarterly Progress Report, January - March, 1995). A final publication on the work entitled "High incidence of *Aspergillus flavus* and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis" is In Press, Mycopathologia, 1996.

Another major project is to identity native plants which might suppress Aspergillus growth and/or aflatoxin contamination of peanuts. Attempts have been made to screen six plant products for their preservative properties regarding peanut storage. Experiments on this subject are described below. Preliminary results obtained in this study were presented during the 5th regional groundnut workshop for West and Central Africa, Nov. 18-21, Accra, Ghana.

USA

Collaborative work with Dr. Olin Smith and graduate student Yolanda Lopez have demonstrated that the use of the norsolorinic acid mutant (red color) may give superior results than a wildtype aflatoxin producing strain of *Aspergillus* in analyzing aflatoxin contamination of peanuts. A publication has been submitted to Crop Science.

Continued cooperative studies with Dr. Thomas H. Adams (Department of Biology, Texas A&M University) have resulted in identification of the entire sterigmatocystin (ST) pathway of *Aspergillus nidulans*. Evidence shows that the sterigmatocystin pathway is functionally equivalent to the aflatoxin (AF) pathway in *A. flavus* and *A. parasiticus* and that this ST/AF toxin pathway is conserved as a cluster of ST/AF genes. Most importantly, the regulatory gene, *aflR*, is found to regulate both the sterigmatocystin and the aflatoxin pathway in all three *Aspergillus* spp. Recent progress has identified the likely site of AflR binding to other genes in the ST/AF cluster. We have also found that external pH regulates the biosynthesis of ST/AF and it is likely that the *Aspergillus* pH regulator, PacC, has a major effect on AF/ST biosynthesis.

Continued characterization of the genes for trichothecene biosynthesis in *Fusarium sporotrichioides* and *F. sambucinum* has shown that the positive regulatory gene Tri6 has retained 91% protein homology and 88% DNA homology for 91% of the protein coding region. Similarly, the putative Tri10 gene ORF1 and ORF2 show, respectively, 80% and 88% protein homology and 86% and 87% DNA homology. Some, but not all of the DNA integrations achieved by homologous recombination between different fragments of the Tri10 region resulted in the alteration of toxin production.

GOALS

The goals of the mycotoxin management project are to develop and apply techniques which reduce the severity of the mycotoxin contamination problems within the Lesser Developed Countries and the United States. Prevention of mycotoxin contamination through the development of aflatoxin resistant cultivars, effective biocontrol strategies, and improved production, harvest, storage, and processing procedures will help reduce the costs of excessive processing to remove the mycotoxins. We will utilize the most appropriate technologies from biotechnology to cultural practices as best fit. For those peanuts which still contain mycotoxins the goal is to develop improved inspection and diversion procedures, and clean-up and detoxification procedures, which will reduce the levels of mycotoxins present within peanut and peanut products to assist in meeting governmental health and safety standards.

OBJECTIVES

1. Investigate the genetic and molecular mechanisms that control mycotoxin biosynthesis in *Aspergillus* and *Fusarium* spp.
2. Develop new assays and studies to assess the importance of other mycotoxins and mycotoxigenic fungi in peanut.
3. Assess Ghanaian plants and plant extracts for their ability to decrease *Aspergillus* growth and/or aflatoxin production.
4. Identify single gene products which inhibit *Aspergillus* growth and/or aflatoxin production and use them to transform peanut plants.

ORGANIZATION AND PERSONNEL

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

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Co-Principal Investigators

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Cooperators

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Dr. Charles Simpson, Department of Soil and Crop Sciences, TAMU, CS

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Ms. Tiffany Sanford, Department of PPM, TAES, TAMU, CS

Graduate Student

Mr. Andy Tag, Department of PPM, TAES, TAMU, CS

Post-Doctoral Research Scientist

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Budget Analyst

Ms. Judy Owens and Mr. Rob Koch, TAMU Research Foundation

Institutional Representatives

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Ghana: University of Science and Technology (UST)

Principal Investigator

Dr. Richard T. Awuah, Department of Crop Science, UST, Kumasi, Ghana

Ghana: Food Research Institute (FRI)

Ms. Kafui Kpodo, FRI, Accra, Ghana

APPROACH

Determine the molecular mechanisms that control mycotoxin production by utilizing the techniques of molecular biology and genetics to identify and clone the structural and regulatory genes required for toxin biosynthesis in *Aspergillus* and *Fusarium* species.

Using microbial assays to identify cultural conditions which may inhibit or induce ST/AF production in *Aspergillus* spp. Identify Ghanaian plants which inhibit *Aspergillus* growth and/or ST/AF production. Evaluate and further develop mycotoxin detection systems and detoxification methodologies for mycotoxin contaminated peanut and peanut products. Further assist, train and encourage host country scientists in conducting, developing and establishing their own mycotoxin research programs and in attaining project goals, including the transfer and adaptation of technologies developed in conjunction with this research project.

RESEARCH ACCOMPLISHMENTS

Texas

<u>Use of microbial assays in evaluation of peanut cultivars and genotypes for Aspergillus colonization and aflatoxin production</u>

The use of *Aspergillus* aflatoxin mutants which accumulate colored intermediates in the aflatoxin pathway is established. The norsolorinic acid - red color - mutant has been most extensively utilized and we have recently

created a norsolorinic acid producing *A. nidulans* strain (we have both *A. flavus* and *A. parasiticus* norsolorinic acid mutants). A collaborative study with Ms. Yolanda Lopez and Dr. Olin Smith (Dept. Soils and Crop Science) has resulted in a submitted paper on this aspect.

Inducing and Inhibitory Environments for Aflatoxin Production

Use of the norsolorinic acid mutant have also been helpful in identifying pH as a regulatory factor in ST/AF biosynthesis. Acidic conditions favor ST/AF production and ST/AF gene expression. Alkali pH repressed biosynthesis of both mycotoxins. This work was conducted in the laboratory, attempts will be made to introduce this concept into storage conditions in Ghana.

Molecular biology and genetics of mycotoxin production

In a collaborative project, Dr. Keller and Dr. Adams reported that the ST genes are functionally and structurally conserved to AF genes in *A. flavus* and *A. parasiticus.* The progress in this molecular project significantly contributes to the Peanut CRSP project; eventually any inhibitors/inducers of ST/AF will be assessed for their effect on specific genes in the aflatoxin pathway. We have likely identified the DNA binding site where AflR binds to other genes in the ST/AF pathway. AflR is an AF/ST pathway specific regulator and is absolutely required for AF/ST biosynthesis. We are currently looking for those genes that regulate AflR. One of these genes may be PacC, a major pH regulatory gene. Putative AflR and PacC DNA binding sites are located close together in promoter regions of ST/AF biosynthetic genes.

Comparison of the organization and DNA sequence of the gene clusters for trichothecene biosynthesis in F. sporotrichioides and F. sambucinum has shown that the gene order and orientation have been conserved fro Tri6, Tri5 and Tri10 ORF1 and ORF2. We are continuing to investigate the function of the Tri10 DNA region to determine how it effects toxin production.

Phyllosilicates and Clays

At Texas A&M University, we have further characterized the interactions between diverse phyllosilicate clays and aflatoxin. Our research indicates that the enthalpy of adsorption (chemisorption), ligand specificity and capacity are critical requirements for optimal reduction in aflatoxin bioavailability and efficacy in animals. All aflatoxin adsorbents are not created equal and should be rigorously tested in vitro and in vivo, paying particular attention to their effectiveness in aflatoxin-sensitive animals and their potential interactions with nutrients, etc. It is important to understand that there may be significant risks associated with the inclusion of a nonselective silicoaluminate (or other adsorbents) in food and feed.

Senegal ISRA

No activity was reported.

Senegal ITA

No activity was reported.

Ghana UST and FRI

A paper describing the distribution of contaminated peanut sources in Ghana is in press in the journal *Mycologia* (joint efforts by Dr. Awuah and Mrs. Kpodo).

UST: Effects of Plant Products on Protecting Peanuts from *Aspergillus* contamination.

Below is a description of the experimentation and results of the effects of several plant extracts on *Aspergillus* growth on peanut kernels.

Source of peanut kernels: Peanut kernels were purchased from the local market. Healthy looking kernels were visually sorted out from shriveled and moldy ones and stored in a freezer until needed.

Inoculation of peanut kernels: A NOR mutant (wA$^-$ diploid) of *A. parasiticus* (supplied by Dr. N. P. Keller, Texas A & M University) was used to inoculate kernels as follows: Conidia from a 7-day-old culture of the fungus growing on chloramphenicol (500 ppm) potato-dextrose agar (CPDA) plate were harvested in 200 ml sterile distilled water and adjusted to 13 x 10^4 conidia/ml. One-hundred milliliters of the conidial suspension was atomized onto 500 g healthy-looking peanut kernels. Inoculated kernels were thoroughly mixed by hand and incubated for two days in a dark enclosure at 24-28°C. The Colony forming units (CFU) of the NOR *A. parasiticus* was then determined by dilution plating on CPDA. The moisture content of the kernels also was determined gravimetrically.

Treatment of peanut samples with plant products. Plants and their products used for preserving peanut kernels were *Cymbopogon citratus* (D.C.) Staff (crude leaf oil), *Ocimum gratissimum* (L.) (dry leaf powder), *Xylopia aethiopica* (Dunal) Rich (dry fruit powder), *Syzigium aromaticum* (L.) Marr & Perr, (clove powder), *Citrus aurantifolia* (Christm.) Swingle (fruit peel oil) and *Cymbopogon nardus* (L.) Rendle (leaf oil). Oils from *C. aurantifolia* and *C. nardus* were obtained from commercial dealers but the rest of the plant specimens were obtained from the market or backyard plantings. When a plant product was used in a powdered form, 30 g of the powder was thoroughly mixed with 150 g of infected peanut kernels (20% concentration) and placed in a high density polyethylene bag (16 x 16 x 22 cm) and sealed with a Difco polyethylene bag sealer, leaving a head space of about 11 cm. The bag was then enclosed in another bag (27x27x46 cm) made of interlaced polyethylene fabric and stored in a dark enclosure (24-28°C). When a plant oil was used, 10 ml of the oil was soaked in a 2.5 g cotton wool ball and stored with 150 g peanut kernel as above. Three replicate bags per plant product were prepared. Peanut samples without any plant product served as the control.

Mycological assay of stored kernels. After 4 and 11 months of storage, peanut kernels from each storage bag were assayed for fungi as follows. A 20 g representative sample was homogenized with 100 ml sterile distilled water at low speed in a Waring blender. An aliquot (0.2 ml) of the initial suspension was transferred with Eppendorf microliter pipette into 10 ml sterile distilled water blank. One millilitre of this final suspension was plated on CPDA plates and incubated on a laboratory bench (24-28°C). Fungal colonies were counted and expressed as CFU/g peanut sample. Only colonies of the *A. parasiticus* NOR mutant were counted at 4 months but all fungi were counted at 11 months. Fresh peanut kernels not included in the assay at 4 months were included at 11 months.

Other data recorded on stored kernel samples after 11 months storage included (1) Embryo and cotyledon discoloration, (2) Presence or absence of fungal growth and sporulation within kernels, (3) Kernel crispness, (4) Presence of wrinkles on the testa, (5) Departure of testa from original color, (6) Scent of plant product picked up by kernels, and (7) Presence or absence of surface mycoflora.

Results and Discussion

By two months after storage, fungal growth was visible on the untreated control kernels as well as those receiving the *C. nardus* oil treatment. By 11 months, all treatments except the *S. aromaticum* and the *O. gratissimum* treatments were associated with varying degrees of fungal growth on the samples. In this regard, the least effective treatment was the *C. nardus* treatment which was similar to the untreated control. These two treatments also were similar to each other with respect to internal kernel discoloration and fungal sporulation. The most promising of the plant products tested was the *S. aromaticum* clove powder which was able to preserve groundnut kernels in a mycologically wholesome condition during the 11 month storage period. Kernels treated with this plant product were similar in several observable grain quality parameters as freshly harvested peanut kernels.

The population of the *A. parasiticus* NOR mutant generally decreased (from an initial value of 2925 CFU/g sample) in all the treatments but the lowest value 20.8 was associated with the *S. aromaticum* treatment (Table 1). The fungus may have lost its competitive capacity to survive on peanut during long term storage due to mutations (e.g. the white spore color?, the NOR mutation?). This strain was completely absent from all the

peanut samples (except those treated with the *C. nardus* oil) after 11 months of storage and other fungi (other *Aspergillus* spp., *Penicillium* spp and various unidentified spp.) were isolated instead. The die-off rate of the *A. parasiticus* NOR mutant must, therefore, be determined to serve as a guide to workers using the fungus in various studies bordering on peanut storage.

High fungal populations were generally associated with kernels stored with the various plant products except the *S. aronaticum* powder which suppressed total fungal population to 7,100 CFU/g peanut sample, a value similar to the 12,200 CFU/g associated with the fresh peanut kernel sample (Table 1).

Even though it has not been possible at the moment to determine aflatoxin levels associated with the stored peanut sample, these results are encouraging. The conviction is that by suppressing fungi (especially the aflatoxigenic ones) on peanut during storage, production of aflatoxins also will be inhibited. It is recognized that the low moisture content of the kernel used in the present study (5.7%) is not ideal for aflatoxin synthesis. Therefore, experiments are in progress to further test the *S. aromaticum* clove powder and probably the *O. gratissimum* leaf powder under near-farm storage conditions (e.g. larger storage sample sizes; two types of storage containers viz. (1) high density polyethylene bags and (2) interlaced polyethylene bags; high moisture maize) using a wild type aflatoxigenic *A. parasiticus* (SRRC 143 SU-1) supplied by Dr. N.P. Keller of TAMU. In this study, aflatoxin levels of the stored products will be monitored. Optimal plant powder concentrations for storage also will be determined. Preliminary results from another on-going experiment indicates that plant powders necessarily must be thoroughly mixed with the peanut kernels for optimal results. Release of plant product fumes into the storage environment is not as effective in inhibiting fungal growth.

Table 1. Effect of various medicinal/culinary plant products on the growth of *Aspergillus parasiticus* (NOR mutant) and total fungal micro-flora on groundnut kernels in storage.

	CFU/g groundnut sample	
Plant products	4 mn storage[1]	11 mn storage[2]
Lime peel oil	145.8	511,000
Ocimum leaf powder	116.7	890,333
Citronella leaf oil	812.5	1,250,000
Xylopia fruit powder	875.0	911,000
Syzigium clove powder	20.8	7,100
No treatment (control)	1,291.7	1,250,000
Fresh kernels (control)	-	12,200
L.S.D. (P=0.05)	362	39,434

[1] Only colonies of *A. parasiticus* were counted.
Initial CFU/g before treatment = 2925.
[2] Except for the citronella oil treatment, figures represent CFU of total fungi/g sample. With the citronella treatment, the figure represents CFU of only the NOR mutant.

PUBLICATIONS AND PRESENTATIONS

Publications

submitted

Lopez Y, Keller N P, Sarr, B, Phillips T D, Cuero R G, Smith O D. Use of colored *Aspergillus* norsolorinic acid mutants to visually screen peanut lines for aflatoxin production. Crop Science

Keller N P, Nesbitt C, Sarr B, Phillips T D, Burow G B. pH regulation of sterigmatocystin and aflatoxin biosynthesis in *Aspergillus* spp. Phytopathology *in press*

Awuah R T, Kafui K. High incidence of *Aspergillus flavus* and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis" Mycopathologia *published*

Yu J, Butchko R A E, Fernandes M, Keller N P, Leonard T, Adams T (1996) Conservation of structure and function of the aflatoxin regulatory gene *aflR* from *Aspergillus nidulans* and *A. flavus*. Curr Gene 29:549-555.

Keller N P, Segner S, Bhatnagar D, Adams T H (1995) *stcS* encodes a putative P450 monooxygenase needed for the conversion of versicolorin A to sterigmatocystin in *Aspergillus nidulans*. Appl Environ Micro. 61:3628-3632.

Presentations and Workshops

Dr. N. Keller attended the USDA Aflatoxin Elimination Workshop end of October 1995 in Fresno, Calif.

Dr. N. Keller attended the Gordon Fungal Biology Conference in June.

TRAINING: DEGREE AND SHORT-TERM

Dr. Mary Fenandes, a post-doctoral research fellow, was partially employed by USAID funds and her work has contributed to identifying *aflR* as a conserved aflatoxin regulatory gene.

NCS/IM/TP

Management Of Arthropods On Peanut In Southeast Asia

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Khon Kaen University
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Virginia Ocampo, Principal Investigator, UPLB, Philippines
Eliseo Cadapan, Principal Investigator, UPLB, Philippines

INTRODUCTION

New peanut price support legislation, consumer concerns over pesticide use in the U.S., increasing foreign competition, and increasing demand for a high protein food source produced economically in developing countries demands continued research focused at low-input, sustainable systems. The research objectives of this project integrate past objectives toward reduced pesticide use practices in peanut production and are the result of world-wide efforts to meet the objectives of integrated pest management and sustainable agriculture. A strong data base has been developed to reach these goals and is the result of well-balanced and strong basic and applied research components for insect management. A thorough understanding of the biological and economic factors involved is also a significant component of this research. The production of peanuts throughout Southeast Asia is a complex agroecosystem subject to a wide variety of plant stresses as well as certain socioeconomic factors. A rainy and a dry season crop are grown in many areas each year and insect pests can be a limiting factor for peanut yield and quality. The management of several insect pests pose a significant challenge for peanut producers. The whole plant may be attacked by one or more species and the plant is exposed to potential insect feeding virtually the whole growing season However, soil insects provide the greatest management challenges. Not only do these pests feed directly on the harvested unit, the pod, and result in considerable crop loss, but the subterranean nature of the pests renders them difficult to detect and manage. To effectively manage these pests in an economically and environmentally-sound approach lending itself toward sustainable agriculture, a sound biological base must be established. Research needs for all insect pests include ecological studies, surveys of pest problems, damage assessments and thresholds, population monitoring and scouting techniques, off-target impacts of pesticide use and potential interactions. A critical component of the sustainable production package is the development and implementation of alternative control strategies including host-plant resistance, cultural and biological control. Such broad-based research programs are difficult due to the field nature of the research, the breadth of the problems to address, and the resources required to effectively conduct such efforts. The information that results, however, helps build effective integrated pest management programs and moves peanut production closer to the goal of a sustainable production system for peanut production in all countries.

IMPACTS

Significant progress has been made in both the U. S. and in developing countries in the develop, use, and integration of cultural practices, alternative control strategies, and host-plant resistance. However, pesticides continue to play a major role for managing insect pests in peanuts in many countries. In the United States and

in Southeast Asia, pesticide use is considered an essential tool for preventing significant crop loss from foliar feeding pests. These situations require an improved system to aid the grower decision making process for insecticide use to limit the indiscriminant use of pesticides while seeking more sustainable, alternative means of pest control. Information on thresholds and biorational control approaches has been developed and put into use in the U.S., the Philippines and Thailand. Studies have documented the benefits of well-timed treatments based on thresholds and the use of biological materials such as *Bacillus thuringiensis*, neem seed extract, and NPVs. Growers in Southeast Asia countries are slowly adopting the threshold concept as part of IPM and accepting the use of prescriptive pesticide use rather than calendar-type sprays.

The past emphasis on host plant resistance in the Philippines and Thailand including more than 10 years evaluating varietal tolerance and resistance of new genotype entries is now beginning to offer potential for actual deployment. Cultivars with more tolerance or resistance (including currently recommended varieties) are being evaluated in IPM programs grown in the actual production areas to test their overall role as integrated into a complete management system. The beneficial effect of natural enemies and inundative releases of parasites has been documented in several areas of the Philippines and proven quite successful and economically sound. In addition, research in Thailand has recently worked toward developing a clearer understanding of the ecology and impact of soil insects on on peanut and the relationship to aflatoxin contamination. Efforts there have also produced a clearer understanding of the relationship between various sucking insects and the presence of certain virus diseases that they vector. Researchers in both the Philippines and Thailand have continued to develop crop loss assessment data for several pests despite the inherent difficulty associated with the development of such information.

The move in North Carolina toward reduced inputs, including minimizing pesticide use, has been frustrated by the arrival of new pest problems, such as tomato spotted wilt virus which is vectored by thrips. However, peanut growers have dramatically improved their ability to use prescription applications of pesticides for certain pests. This includes the use of more environmentally-sound pesticide selection and more efficient timing of application. These improve control strategies generally reduce overall pesticide use and reduce costs while preserving crop yield and quality. Two empirical models for predicting pest outbreaks continue to prove useful in alerting growers to potential pest outbreaks are being used in conjunction with the leafspot advisory program in North Carolina. Recent collaborative efforts with non-governmental organizations (RAFI, USA), growers' groups (North Carolina Peanut Growers Assoc.), and N. C. State University are leading to grower research and demonstration projects, overseen by the Peanut CRSP PI to evaluate the deployment of sustainable agriculture practices.

North Carolina growers continue to use the economic thresholds for thrips, corn earworms, and leafhoppers in peanut IPM programs. These allow growers to avoid some pesticide applications and to make more intelligent decisions in light of the potential threat of tomato spotted wilt virus vectored by thrips. The use of alternate, less toxic products for thrips management has been demonstrated to have economic as well as environmental advantages. Extensive field testing of 80 different entries in a host-plant resistance has provided promise for new opportunities in insect management using resistant germplasm. Evaluations included thrips and southern corn rootworm feeding injury. Further confirmation of the importance of thrips and virus overwintering is leading to additional studies toward developing a management approach for this problem. Significant varietal affects on virus incidence in the host-plant resistance plots and trials utilizing currently available cultivars indicate potential for the use of varieties to aid in an overall management strategy for this serious problem.

MAJOR ACCOMPLISHMENTS

PHILIPPINES - Reduced funding in the final year of the project in the Philippines limited the overall program and objectives were limited to continued host-plant resistance evaluations and IPM systems evaluation, The overall research program in the Philippines has made outstanding progress in evaluating host-plant resistance,

biological control, alternative management strategies, developing economic thresholds, and pest biology. The progress seen in the host plant resistance work has developed an excellent data base evaluation under Filipino production systems. The principal investigators have taken a critical step forward in the final deployment of IPM practices are testing the integration of this information into IPM programs in the various production regions. Studies sites evaluating these IPM systems have included underway in the Cagayan Valley, near Pamapanga, and in Mandaue City. These trials have shown that IPM is a viable alternative to standard "farmer managed" plots and conventional insecticide use is often avoided. These studies have looked at current Seed Board recommended varieties and promising cultivars used in conjunction with classical biological control programs.

Biological materials such as *Bacillus thuringiensis* have proven very effective against a complex of defoliators. These studies and those on the effects of intercropping and fertilizer use have contributed greatly to the development ofeffective IPM programs.

The continuation of studies validating the use of biological control programs using *Trichogramma* sp. and a microbial insecticide *Bacillus thuringiensis* shown promise for future wide-scale implementation. Biological control studies have shown practicality and profitability using an integrated program in IPM evaluation trials.

THAILAND - Research continued to place primary emphasis on the evaluation of germplasm for insect resistance at both DOA and KKU. Studies have focused on the development of a variety of control strategies, including host plant resistance, for IPM programs. Since some farmers use insecticides, one objective included basic insecticide evaluations to improve the timing of application to increase yield and look at the total integrated program using host plant resistance as well as cultural and biological control methodologies. Studies on the relationship of virus incidence and transmission by insect vectors has been slow, but making steady progress. These studies enhance our understanding of the dispersal of several insect pests and the risk of disease transmission.

More recently studies in Thailand have included research investigating relationship of pod-feeding insect pests and the incidence of aflatoxin. Recent surveys have documented the widespread nature and seriousness of soil insect pests of peanuts in Southeast Asia therefore this work is critical and long overdue in developing a complete understanding of crop loss assessment. However, research on soil insects is tedious, labor intensive, and slow to provide documentable results. It is also important in future aflatoxin elimination management strategies.

NORTH CAROLINA - Our emphasis continues to focus on the development of a sustainable agricultural system for peanuts in the U.S. Increased effort to minimize preventive pesticide use and to integrate biological and cultural control practices have made significant progress. Coordination with growers organizations and NGOs has established a working relationship for enhanced on farm validation of this research. The validation of forecast models for the lesser cornstalk borer and spider mites has proven to be an invaluable tool for pest management. Evaluations of pesticide interactions are increasing our understanding to reduce overall pesticide dependence, minimize the threat from tomato spotted wilt virus, and minimize secondary pest outbreaks.

New studies focusing more on the overwintering of the thrips and the tomato spotted wilt virus indicate the virus can overwinter in thrips and be transmitted to host plants immediately in the spring. This research helps determine virus/vector relationships with the increasing threat from tomato spotted wilt virus in peanuts which is vectored by these thrips. Studies looking at varietal difference in the incidence of this virus indicate commercially available cultivars such as NCV-11 have less tomato spotted wilt virus than other varieties such as NC-9. These studies seek to determine if these differences are the result of inherent tolerance or resistance to the virus by the plant or some effect on thrips feeding behavior by different cultivars. Upon completion these studies will dramatically improve our understanding of the vector and help to manage this disease. These studies complement past vector/virus relationship work in Thailand. The host plant resistance work has been

further expanded and has already proven beneficial in identifying resistant germplasm for both foliar and soil insect pests. In addition, this research has revealed rather dramatic differences in the incidence of tomato spotted wilt virus among the various genotypes.

GOALS

The major goal of this project has been to develop an integrated and sustainable management system for those arthropod pests that limit peanut production.

OBJECTIVES

1. To continue the evaluation of a wide variety of genetic material for possible insect tolerance or resistance to single species and arthropod complexes.

2. To develop damage assessment data for the arthropod complex and relate to host plant phenology. This information will be incorporated into economic thresholds for use in IPM programs.

3. Study the biology and ecology of the important arthropod pests. This includes studies on virus/vector relationships. Conduct surveys to determine abundance and pest status of arthropods.

4. Determine the effect of cultural practices (i.e., tillage) on insect populations and host plant damage.

5. Utilize monitoring devices to gain a better understanding of insect biology and use as a predictive tool for insect occurrence and pest abundance.

6. Develop an effective IPM program and demonstrate the benefits through research findings and on-site demonstrations utilizing information developed from the Peanut CRSP in North Carolina, Thailand, and the Philippines.

7. Evaluate the potential for biological control as a realistic approach to arthropod management in peanuts.

ORGANIZATION

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	Technicians:	Mr. Peter T. Hertl, Dept. of Entomology
		Mr. Brian Royals, Dept. of Entomology
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	Graduate Students:	Mr. Rob Hummel, Dept. of Entomology
		Mr. Lloyd Garcia, Dept. of Entomology
	Cooperators:	Dr. Tom Isleib, Dept. of Crop Science
		Dr. Tom Stalker, Dept. of Crop Science
		Dr. Jack Bailey, Dept. of Plant Pathology
B.	Counterpart Institution:	Khon Kaen University, Khon Kaen, Thailand
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C.	Counterpart Institution:	Department of Agriculture, Field Crops, Bangkok, Thailand
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E.	USAID Project Officers:	K. A. Rushing/Manila, Philippines

APPROACH

The main objectives and goals of the Principal Investigators in the Philippines, Thailand, and North Carolina continued the emphasis on potential resistance from the various peanut genetic material under trial. However, due to reductions in funding during this final year the overall research effort was somewhat reduced from previous years, although H.C. PIs provided alternate support to help bring numereous projects to fruition. These genotypes evaluated in several locations within the peanut-producing regions in addition to research station sites to provide data under a range of agronomic, environmental, and insect pest conditions. Since multiple species of insects attack peanuts in Southeast Asia, the search for resistance to insect guilds rather than a single species has been emphasized. Thus broad resistance would probably result in a more sound release for growers, since the off-target effects of host plant resistance on related pest species is not well understood. After many years of testing with varying levels of pest pressure and different environmental factors several entries have shown promise when tested in actual farm situations. The more suitable entries are being tested in advanced IPM trials to determine the cultivars that show the best fit into such systems for the most integrated overall approach. In North Carolina, the host-plant resistance effort was further expanded in cooperation with Dr. Tom Isleib with renewed effort on foliar feeding and soil insect pests. These trials also investigated the relationship between thrips abundance and feeding to the incidence of tomato spotted wilt virus among different genotypes.

Surveys of all arthropod pests, especially subterranean ants and other soil pests, have demonstrated the importance of these problems on an annual basis in Southeast Asia. These studies are enhanced with the addition of studies on the biology, ecology, and the management of the subterranean pests and its relationship to aflatoxin contamination. Additional research on sampling and traditional management approaches is critical for soil pests. Very little is know about crop losses to these and other pests. Insect abundance and plant damage relationships are being refined through the continuation of crop loss assessment studies. New efforts on developing a better understanding of the relationship of thrips movement and overwintering and the incidence of the tomato spotted wilt virus continue at an increasing pace. Related studies on vector and virus relationships have been conducted in Thailand. Traps to monitor migration, as well as studies of feral host plants, insect abundance in field, and disease incidence have been useful in understanding vector management

considerations. The continued increase in the incidence of tomato spotted wilt virus in the North Carolina peanut crop each year has justified a more significant effort investigating the role *Frankliniella fusca* laying in vectoring this disease in peanut. In North Carolina, these studies focus exclusively on thrips, while in Thailand, studies include aphids and jassids as well. The effect of varieties on the incidence of the disease has been examined and demonstrated to be of potential for the development of management strategies for this disease in North Carolina.

The demand for low input systems worldwide and reduced pesticide use in the U.S. encouraged a continuation of studies on the use of biological control agents in the Philippines and North Carolina. These studies will include the release of egg parasites, *Bacillus thuringiensis*, nuclearpolyhedrosis virus, and entomogenous nematodes, with the ultimate goal of incorporating these into a totally integrated control system. Since pesticides continue to be an important tool in the management of insect pests of peanuts, studies looking at the off-target effects of pesticides will be continued in all three countries to determine the detrimental effects and the potential to induce secondary pest problems. New collaborative linkages with organizations and farmers in North Carolina pursuing on-farm sustainable

The past six years of research have developed the essential components of an Integrated Pest Management program in each respective country. Each of these programs vary significantly in their comprehensiveness and management tools used, but each forms a bridge toward a more sustainable approach. Further research is targeted toward more refined programs. Due to the changing emphasis within USAID and the Peanut CRSP, the overall objective in Thailand was to bring together the findings from the many years of research and system validation and begin wide-scale implementation through the Department of Agriculture Extension. Furthermore, the research expertise and accomplishments will be directed to function as a regional center of excellence for peanut pest management in training scientists from surrounding countries throughout Southeast Asia. The Thai scientists also produced a publication that provides a complete overview of IPM research findings and strategies for farmers and extentionists.

ACCOMPLISHMENTS IN DETAIL

PHILIPPINES - As in past years Drs. Ocampo and Abilay continue to coordinate the host-plant resistance research effort and this has proven mutually beneficial and resulted in an excellent joint program and efficient use of funding. The data base developed by Dr. Ocampo has provided an excellent overview of the resistance available for the pest species in the Philippines. The recent expansion of this program to the research station in the Cagayan Valley region has been a much need effort to further validate the usefulness if these genotype, however the end of the Peanut CRSP project severely limits the opportunities to further test potential germplasm in the actual production areas and will force a limited program to continue on site at UPLB. Dr. Cadapan focused on limited genotype evaluations did include the use of various IPM strategies across certain cultivars at several regional sites. Work continued on appropriate insecticide and intercropping peanut with banana and other crops as part of a more sustainable and integrated system.

As has been reported in previous years, the data on host plant resistance were quite extensive and too voluminous to present in tables within this report. In addition, a rather wide range of insect preference tolerance, and/or resistance exists among the various entries and this varies from year to year based upon local environmental conditions and insect pest pressure. However, several entries that have been evaluated over several years show excellent promise for incorporation into an IPM program. The level of resistance available does, however, appear to offer an adequate level of protection under normal pest pressure. Overall pesticide use by the Institute of Plant Breeding has been reduced and Dr. Abilay's program has much better information on insect resistance. During the Peanut CRSP program, UPL Pn 10 and IPB Pn 2 were released and these have all been studied for insect susceptibility/resistance through this collaboration prior to release.

Work in Quirino to evaluate the intercropping of peanut and banana has provided significant information over the past few years. Perhaps the most important component has been the dramatic differences in yield and insect performance in comparing trials at UPLB with the locations in the North. The farmers in this area still prefer BPI Pn 9 despite its known susceptibility to insects. While varieties like UPL Pn 6 performed well at UPLB, its performance lagged behind BPI Pn 9 at tests in Quirino. In addition, differences in the level of insect damage occurred in simulated shading to mimic intercropping practices

Dr. Cadapan continued work with *Trichogramma* sp. and with *Bacillus thuringiensis* in peanut with the major impact being that recommended cultivars evaluation for local needs are based on results of conventional and IPM approach management strategies in both the wet and dry seasons. In addition, demonstration plots have been used to educate farmers concerning the use of IPM on peanuts. Data for one newly-released variety(UPL PN 10) under farmer (conventional) and IPM systems have proven ideal for enhancing yield and net profit.

ADDITIONAL STUDIES IN THE PHILIPPINES-Studies on the subterranean ant have not been pursued due to limitations in funding and the termination of the Peanut CRSP project. Current knowledge indicates that poor crop stands are often directly related to this pest and this pest deserves additional attention.

THAILAND - Drs. Manochai Keerati-Kasikorn and Turnjit Satayavirut focused exclusively on the finalization of research data from the past six years of research. The ultimate goal of this final year was to assemble the data into a format for validation as integral components of an effective IPM program by extensionists. Only limited research was conducted on Peanut CRSP funds during 1995-96. However, the field screening during wet and dry seasons from four experiments including the F-Test, IGR-Test, ICRISAT-Test, and the LHT-Test were conducted for *Heliothis*, Thrips, and jassid injury at the Khon Kaen Field Crop Research Center, Prabuddhabat Exp. Stn., and the Rayong Field Crops Research Stn. The studies found differences in insect injury among several entries, but also found this varied with the year, the season, and the location. After Many years of evaluations approximately 145 lines were sent to Dr. Peaingpen Sarawat, an agronomist at the Khon Kaen Field Crops Res. Center for the germplasm collection and or his research in peanut characterization.

Research on the influence of soil insect pest damage and the incidence of aflatoxin contamination was also continued. Field collected pods damaged by a complex of soil insect pests (ie. subterranean ant, *Dorylus orientalis* Westwood; termite, *Odontotermes* sp.; and white grub, *Maladera* sp.) were categorized according to different levels of damage. Pods and seeds were tested for *A. flavus* contamination in the laboratory using AFPA. The incidence of the fungus was determined 5 to 7 days after placing the pods or seeds on the media. Results indicate that the percentage of infected pods and seeds increased in relation to the increase in damage from insects to the pod or seed (Table 1). Cultivars also affected the incidence of aflatoxin with Tainan 9 having a higher incidence and peanuts grown during the dry season also has a higher level of contamination.

Table 1. Percentages of *Aspergillus flavus* - type fungi on Tainan 9 peanut variety pods and seeds damaged at various levels by soil pests at Prabuddhabat Experiment Station.

% *A. flevus* Infection Treatments	Pod [1/]	Seed 2/1
1. Good pod	2.08 c	2.00 c
2. 1.25% EPS[4/]	5.56 c	8.00 c
3. 26-50% EPS	21.10 b	8.00 c
4. 51-75% EPS	X	X
5. 76-100% EPS	X	X
6. PP 1 hole, PS 1 hole5/	62.96 a	52.00 b
7. PP 2 holes, PS 1 hole	X	X
8.PP 1 hole, PS 2 holes	45.83 a	32.00 b
9. PP 2 holes, PS 2 holes	48.15 a	9.00[3/]

% C.V.	32.28%	56.74%

[1]average of 6 replications
[2]average of 5 replications
[3]discard from statistical analysis (1 replication); X = no experiment
[4]external pod scarification
[5]PP = penetrated pod, PS = penetrated seed
Within a column, means followed by the same letter are not significantly different a 5% level of DMRT.

Some of the potentially most valuable information emerging form this research that was assembled during this final year is that of documenting insect damage and the benefit (or lack of benefit) from the use of insecticides for sucking insect pest control. While there were differences in yield among cultivars and insecticide use reduced insect damage, the incidence of virus, and increased yield, there was not an increase in profit. In fact summary results indicate the most profitable production scheme when only sucking insect pests are present is no insecticide use (Table 2).

Table 2. Market prices of insecticides, cost of control and the profit of each treatment for control insect pests of peanut, 1994.

Treatment	No. of Sprays	Cost of Control (Bahts/rai)	Profit (Baht/rali) Dry	Rainy	Average
1. E-R2	3	180	-160.8	-175.7	-168.3
2. R2-H	4	240	-282.4	-304.7	-293.6
3. Sucking Ins. Cont.	7	420	-411.8	-353.9	-382.9
4. T1 + T3	10	600	.572.6	-542.9	-557.8
5. T1 + T2 + T3	11	660	.596.6	-698.6	-647.6
6. T1 + T2 + T3 +Bt.	14+6 (B.t)	840+648	.1,354.0	-1,508.7	-1,431.4
7. Check	--	--	+312.0	+525.3	+418.7

Insecticides	Price Baht/liter/kg	Cost of Control (Baht/rai)
1. Chlorpyrifos	300	60
2. Carbosulfan	300	60
3. Centari (B.t.)	900	108

[1]Only the cost of insecticides used for application.
[2]By comparing the price from the obtained seed of each treatment with the untreated yield; one kilogram seed + Bahts; (+) = the profit and)-_ = the loss.
T1 and T2 = spray with chlorpyrifos at the rate of 50 ml/20 liters.
T3 = spray with carbosulfan at the rate of 50 ml/20 liters.
Bt. = spray with centari at the rate of 30 g/20 liters.

NORTH CAROLINA-Models for the lesser cornstalk borer and twospotted spider mite were validated during 1996. The lesser cornstalk model predicted early problems from this pest which quickly subsided when significant rainfall occurred.. The spider mite model predicted no outbreaks in July or August due to the cooler and much wetter than normal conditions. The integration of these models into an already existing system for peanut leafspot forecasting has greatly facilitated their implementation and adoption. County agents are using this information and relaying it directly to growers to reduce unnecessary pesticide use, refine current control strategies, and help target cultural practices to minimize the impact of these pest.

The Tomato Spotted Wilt *Tospovirus* (TSW) first began to appear in North Carolina peanuts during the early 1990's. Since this time the virus incidence has remained below 1% overall in production fields. However, an increased incidence of TSW in scattered locations in 1995, and the historic incremental increases in the disease incidence in Texas and Georgia indicate that this disease poses a serious threat to peanut production in North Carolina. To better understand the epidemiology of the disease in North Carolina peanuts, research was begun in 1992 to determine the spring movement of the TSW thrips vector, *Frankliniella fusca* (Hinds), the tobacco

thrips, into peanuts; the overwintering potential of viruliferous tobacco thrips; and the identification of TSW tolerant peanut genotypes.

When this research was begun little was known concerning the life history of tobacco thrips, the presumed principal vector of TSW in North Carolina peanuts. Studies of the movement of tobacco thrips into spring planted peanut fields indicated that the thrips were widely distributed at the time of planting, and that migration appeared to be local, not long distance (Table 3). Also, emergence traps placed over newly planted peanuts indicated that tobacco thrips were not present in the soil at the time of planting. More recent findings by Cho et al. (1995) support the findings of Watts (1936) who reported that tobacco thrips overwintered at the base of grasses in South Carolina. Sticky traps deployed 8 inches above the ground in peanut and corn fields during the winter trapped no thrips during the months of January and February, indicating little movement of overwintering tobacco thrips during these months.

Table 3. Cumulative thrips counts from sticky traps deployed in a peanut field for 14 days from day of planting.

***Trap**	1	2	3	4	5	6	7	8	9	10	11	12	13
1992	27	20	26	27	41	42	25	39	37	20	27	37	42
1993	38	23	29	20	20	25	22	28	18	27	35	29	36

*Traps were deployed within a 2 acre peanut field in a cross-hairs, or X/Y, type grid with trap #4 in the center.

Studies with tobacco thrips are being conducted to determine the length of time viruliferous thrips can survive cold temperatures and still transmit TSW. This is an important piece of information from a management perspective. If overwintering thrips can survive to transmit the virus to newly planted peanuts, then the control of overwintering weedy hosts of TSW may not mitigate the incidence of the disease. Current findings indicate that viruliferous thrips can transmit TSW after being refrigerated for six weeks at 4°C. These experiments are experiencing difficulties with secondary infections causing thrips mortality.

An increase in the incidence of TSW in North Carolina peanuts in 1995 has allowed investigations into the presence of TSW resistant or tolerant genotypes to be conducted under North Carolina conditions. Experiments conducted in Texas (Black, 1987) and Georgia (Culbreath, 1992, 1994) documented up to a two-fold difference in TSW incidence in some cultivars. In North Carolina, two varieties, NC-9 and NC-V11, were monitored for symptoms of TSW in two-row plots of 100 feet per row in 1995. The final incidence of symptomatic plants was twice as high in NC-9 compared with NC-V11 (Table 4). Thrips damage was also monitored in the two genotypes, and it was found that NC-V11 had a consistently higher level of thrips damage throughout the season than NC-9 (Table 5).

Table 4. Number of Infected Plants - 91 Days Post-Plant

Variety	**Plot #1**	**Plot #2**	**Plot #3**	**Plot #4**	**Average**
NC-9	9	5	12	n/a	8.67
NC V-11	2	2	3	2	2.25

Table 5. % Damaged Leaflets - 38 Days Post-Plant

Variety	Plot #1	Plot #2	`Plot #3	Plot #4	Average
NC-9	36	28	48	n/a	12
NC V-11	52	44	76	48	55

A replicated field test was conducted to determine the relative resistance or tolerance to TSW of three North Carolina peanut genotypes, including NC-9 and NC-V11 was conducted in 1995. Tobacco thrips occurrence were also being monitored on the three genotypes, but results from this research are not yet available. A concurrent greenhouse study is also being conducted using laboratory reared viruliferous tobacco thrips to determine the susceptibility of the three genotypes to thrips transmitted TSW. It is hoped that this may prove to be a useful technique when evaluating peanut genotypes' susceptibility to TSW.

Host plant resistance work in cooperation with Dr.Tom Isleib, peanut breeder at N. C. State University, was expanded during 1995 at the Peanut Belt Research Station with evaluations for thrips, leafhopper, and southern corn rootworm damage, and tomato spotted wilt virus incidence 80 lines. These studies found significant differences in the level of damage among several entries including the presence of the tomato spotted wilt virus. These data are still under final analysis and will be presented at a later date, but preliminary data indicate some germplasm may possess reasonable levels of resistance to these pest problems. These studies do provide insight into the potential for integrating host-plant resistance more fully into sustainable production systems. The significant differences in the level of tomato spotted wilt virus among different lines is of great significance in light of the inability to directly manage this disease by controlling the vector, thrips. Data on thrips abundance and feeding injury are being compared to level of virus among plots and this analysis is still underway. A Ph.D. graduate student is being recruited to further expand this work in the future.

The southern corn rootworm, *Diabrotica undecimpunctata howardii* Barber is a primary pest of peanut in North Carolina and Virginia and an occasional pest in South Carolina, Georgia, Alabama, and Texas. Currently, no integrated pest management alternative exist for this pest in peanut and management is based solely on preventive application of soil insecticides. Past research using a pheromone to predict rootworm abundance was somewhat successful, but unfortunately the risk as perceived by growers was too great to justify its use on a wide-scale basis. While this research provided great insight into future management strategies, the overall benefit was marginal for growers in light of the intensive sampling required. A risk index has now been developed that integrates factors that influence rootworm and peanut pod damage to estimate level of risk in individual fields, and thus allows for more economical rootworm management (Table 6). Evaluation of the index using field case studies to compared predicted risk to actual percent pod damage showed that 21 of 33 cases resulted in YES ratings, which indicates that in the majority of cases, the index was accurate at predicting general risk to pod damage. Eleven cases received NO ratings which indicate that the index overestimates the pod damage risk. Only one case received an underestimation of risk to pod damage. This risk index is currently being further validated with 12 additional case histories in North Carolina and will be evaluated in cooperation with a regional farmers organization in North Carolina during 1997. This risk index offers the potential for a low risk approach to accurate pesticide use to manage this pest with the potential of reduced inputs for growers(Tables 7 & 8).

Table6. Southern Corn Rootworm Risk Index for Peanut Pod Damage

Peanut cultivar resistance	Value	Field history of rootworm damage	Value
Other	20	High	15
VA 93B	10	Moderate	10
NC 6	5	Low	5

Soil Texture

Loam	15
Fine-sandy loam	20
Loamy sand	5

Planting Date

After May 15	15
April 25 - May 15	10
Prior to April 25	5

Drainage class

Poorly drained	20
Somewhat poorly drained	15
Well drained	5

Total Score	
Less than or 3qual to 40	LOW RISK
45 - 65	MODERATE RISK
Greater than 65	HIGH RISK

Table 7. Southern Corn Rootworm Risk Index Scenarios A

NC 6

Low Risk		Low Risk		Moderate risk	
NC 6	5	NC 6	5	NC 6	5
LS	5	FSL	10	L	15
WD	5	MWD	10	SPD	15
No	0	Low	5	High	15
Apr 20	5	May 5	10	Apr 20	5
Score	20	Score	40	Score	55

Table 8. Southern Corn Rootworm Risk Index Scenarios B

VA 93 B

Low Risk		Moderate risk		High risk	
VA 93B	10	VA 93B	10	VA 93B	10
LS	5	FSL	10	L	15
WD	5	MWD	10	SPD	15
No	0	Low	5	High	15
Apr 20	5	May 5	15	Apr 20	15
Score	25	Score	50	Score	70

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

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INTRODUCTION

Groundnut (peanut) rosette (GR) 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, causing total local crop loss, induces farmers to plant other more dependable crops. Before control measures can be implemented, the source of the rosette virus must be found, the nature of resistance elucidated, and resistance incorporated into drought-resistant, short season peanut.

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. Recent reports 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 in the U.S.,data suggest much higher losses in S.E. Asia and China. The virus infects a sufficient number of plants to have an economic impact on total peanut production.

The incidence of tomato spotted wilt virus (TSWV) infecting peanut has increased in the U.S. in the past decade and has threatened production in a three county area of Texas. Increased incidence in the south eastern states has been noted in 1987, 1988, 1989, 1990, 1994 and more dramatically in 1995. The high yield loss by this virus poses a new constraint for peanut production.

The prominent viruses infecting peanut in Asia are different from those in Africa and the Western Hemisphere. Peanut bud necrosis virus (PBNV)(in the same group as TSWV) causes serious problems in India and Thailand. Peanut chlorotic streak virus (PCSV) has only been reported from India.

Peanut mottle virus (PMV) infects peanut on every continent where peanut is grown. Because it is seed and aphid transmitted, it infects more peanut plants on a global basis than any other virus.

This project was initiated in July 1990 and funds were available in Georgia about three months later. Memorandums of Understanding and Plans of Work were sent to all host countries but signed forms were not received in Georgia until 1991 so funds were not available to host country participants until spring 1991.

IMPACT

Resistance to GR in peanut lines that are acceptable to growers will permit greater production of peanut in Africa. This production will be by the subsistence farmers and provide a more reliable food crop that will enhance their diet. In general it will increase food production across the whole of Africa where peanuts are grown.

Identifying and transferring plant resistance to viruses in peanut and transforming peanut with virus coat protein genes are two systems that have the greatest potential to reduce peanut virus diseases and thus provide a more sustainable crop which will increase the world food supply.

ACCOMPLISHMENTS

This project that started in 1990 had the main objective to transform (move foreign genes into peanut cells) and regenerate fertile plants from those transformed cells. The foreign genes that we are using are the genes from viruses that code for the production of virus coat protein. We have developed two methods that can move genes into peanut.

One method uses enzymes to digest immature cotyledonary tissue to remove the cell walls leaving individual cells without cell walls (called protoplasts). The protoplasts are placed in a solation with the virus coat protein genes and with electroporation (electric impulses) induce small openings in the protoplast. These protoplasts are stimulated to divide when placed in the presence of nurse culture cells. As these protoplasts divide, the cellular nucleic acid can incorporate the coat protein genes into the peanut nucleic acid. Using regeneration techniques, these transformed protoplasts are regenerated into whole fertile plants.

The second method used a bacteria to carry the foreign genes into peanut tissue. The virus coat protein genes are inserted into a binary vector which is incorporated into a strain of *Agrobacterium tumefaciens*. The bacteria is placed on immature leaf tissue which it can infect. After infection the bacteria are killed but they have already carried the foreign genes into the peanut cells. The tissues of peanut are then regenerated and we select the regenerates that have acquired the gene.

We have been successful in producing transgenic plants to TSWV, BNV, PCSV and PStV. The transgenic plants produced seed and these seed, when planted, produced progeny that are normal in all visual aspects. Future work will produce homozygous lines and selected lines with (hopefully) resistance to the invasion of the infectious viruses.

A comprehensive package for virus control of peanut in Asia has been developed (S. Wongkaew - in press). The basic tenents of this package incorporates plant resistance, use of virus free seed and cultural practices such as adjustment of sowing date, using high plant density,and intercropping peanut with fast growing non-host crops.

In Africa, the program has relied on traditional breeding programs for the selection of traits to counteract the effects of virus infection. This program has been highly successful by releasing three varieties (M412.80I, MDR8-19 and K720.78) having resistance to groundnut rosette.

Thus assimilating the accomplishments in Asia, Africa and the U.S. with an additional three to five year research effort should provide sufficient control of virus diseases to lower the constraints of peanut production.

GOALS

The overall goal of this coordinated research program was to develop strategies and control measures to minimize incidence of the major peanut virus diseases, to foster interdisciplinary studies between countries and to develop linkages between supporting food production agencies. This was primarily be accomplished by strengthening peanut production programs in the host countries and the US through training and active research projects.

OBJECTIVES

Host Country - Nigeria

Phindile Olorunju completed her Ph.D. degree at the University of Georgia in virology/breeding. She has carried out a program of breeding for resistance to GR. Currently most African growers would like different resistant lines (RMP-12 has a mottled seed coat, RG-1 is not proper size, etc). Other studies (objectives) were to get GR resistance into a range of season lengths, develop state trials and demonstration plots to assess disease and agronomic performance at a range of sites in Sudan and Guinea Savannah regions and to show farmers that resistant lines prevent epidemics of GR. Other objectives were to multiply seed of resistant released lines so they are readily available to farmers; interact with other Peanut CRSP programs such as the breeding project in Burkina Faso and the ICRISAT Sahelian Center for dissemination of ideas, information and breeding material.

Host Country - Thailand

Techniques for identification and manipulation of the peanut viruses have been established. The virus surveys are completed. Major emphasis has been placed in various avenues that leads to disease suppression or control. Some peanut cultivars have been identified with low rates of seed transmission to PStV and PMV. Studies were directed to determine if this trait is genetically controlled, and if so, to work with plant breeders to incorporate this resistance into acceptable peanut cultivars. A US peanut cultivar, Southern Runner, has resistance to TSWV. This was tested for resistance to BN and yellow spot, diseases of peanut in S.E. Asia. BN and yellow spot are apparent relatives of TSWV. As resistant lines or transformed peanut plants become available from the US project they will be tested for effectiveness in S.E. Asia.

United States

Studies have been completed on the nature of the TSWV epidemics in peanut such as effects on yield, thrips as vectors, virus spread and field screening of commercial cultivars for resistance. This research project also involves the transformation of peanut (Arachis hypogaea) with the DNA sequence coding for the coat protein of several peanut viruses to which a GUS marker gene will be attached.

Organization/Personnel:

A. U.S. Lead Institution:	University of Georgia (UGA)
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Co-Principal Investigator	Dr. Mike Deom Plant Pathology University of Georgia Athens, GA 30602
B. Nigerian Counterpart Inst:	Institute of Agricultural Research (IAR) Ahmadu Bello University, Samaru, PMB 1044 Nigeria
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C. Thailand Counterpart Inst:	Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
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D. Informal Cooperation: ICRISAT, Patancheru P.O., A.P., 502324, India

Principal Investigator: Dr. D.V.R. Reddy

APPROACH

Host Country - Nigeria

Inheritance of resistance studies were conducted in a diallel test with eight peanut genotypes (28 crosses). The studies were designed to determine the nature of resistance to GR, the number of host genes involved in resistance, individual early-maturing F_3 plants with resistance to GR, and F_2 and F_3 plants with acceptable agronomic characteristics. Selection of F_2 and F_3 plants provides the basis for this breeding program which seeks improvement in yield performance and incorporation of new traits such as drought resistance associated with resistance to GR. Screening for resistance to GR in all breeding generations includes both field and laboratory/screenhouse studies. Field screening was done by providing virus inoculum pressure by planting aphid-infected plants in alternate rows with test genotypes. The field inoculum included both GRV and GRAV. Mechanical inoculation with GRV only was done in the laboratory/screenhouse tests. The two methods are useful in identifying interactions between the two viruses that might affect certain agronomic characteristics. Principal investigators (host country and U.S.) maintained communication with Peanut CRSP personnel having breeding programs (Tx/BCP/WA) in other W. Africa countries. Site visits to the breeding plots for selection of virus resistance were made when appropriate.

Resistant (example-RMP-12) and susceptible (example-MK374) peanut lines were increased and given to farmers in diversified areas. These seed were planted as demonstration plots to convince growers of the value of using resistant lines to prevent GR epidemics.

Host Country - Thailand:

Most emphasis was placed on BN since it has been identified as the prominent virus disease in Thailand. These studies included improved diagnostic studies, identification of vectors, virus isolate characterization and epidemiological studies.

Since PStV is so important in other S.E. Asia countries, a geographical area with no PStV incidence the previous year was selected. Plots of approx. 10 m^2 with 20 m spacing between plots were established. Maize was grown as a barrier crop between plots. Spacing within and between rows was approx. 30 cm. Randomly spaced plants (100 in each plot) were inoculated at the 1st true leaf stage with PStV. The infected plants were tagged. Yield data from the mechanically infected plants and the same number of healthy plants were compared in each plot.

To determine virus incidence, a single green leaf was taken from each of 10 plants per acre, at random throughout the field. Incidence per county, state or specified areas was obtained by dividing the total number of plants infected by the total number of plants sampled. To detect other viruses, additional samples were taken from plants with virus like symptoms.

Seed from the U.S. Plant Introduction Unit or the ICRISAT collection were screened for virus resistance in greenhouse and field tests. Field tests were conducted with infected source plants placed in the edge rows and internal plot rows separating test plants. Greenhouse tests were by mechanical inoculation. Non-seed transmission of PMV has been identified in some peanut lines and these lines were tested for non-seed transmissibility with PStV.

United States:

Arachis hypogaea types and 'wild' peanut types were acquired from the U.S. Plant Introduction Station. Seedlings, rooted cuttings and other progeny were mechanically and vector inoculated (separately with TSWV, PStV and PMV) using general virology procedures. Lines showing promise of resistance were field planted and subjected to inoculum pressure from infected source plants. Interspecific lines were also tested by mechanical and vector inoculation.

Primary source of virus was sought by serological tests of weeds and other plants growing in areas where TSWV epidemics have occurred in prior years. Thrips vectors were trapped as they emerge from the soil and serologically tested for TSWV. Plots were devised to test for internal virus spread as opposed to continual virus source from outside the plots. TSWV infected peanut seedlings were transplanted into some plots but not others and comparative incidence in each plot analyzed. Known TSWV source plants (peanuts, datura, tomato or tobacco) were placed in and near healthy peanuts and spread was carefully monitored.

Four approaches for transformation were attempted. The first approach involved electroporation of peanut protoplasts in a solution containing the coat protein sequence for peanut stripe virus. Electroporated protoplasts were plated onto a GUS selection medium and those protoplasts showing incorporation of the coat protein sequence into their genomes, as determined by the GUS assay, were regenerated into complete plants. The second approach involved the direct bombardment of cultured peanut shoot tips or meristems utilizing the particle gun. In this procedure the coat protein sequence is bound to microscopic tungsten or gold particles that are forcibly "shot" into the cells or tissues. Incorporation of the coat protein sequence into the peanut genome is determined by the GUS assay. The third approach placed the coat protein gene in solution with protoplasts and polyethlene glycol to induce protoplast penetration by this gene. The fourth used Agrobacterium to carry the genes into the peanut tissue.

Early success in isolating protoplasts lead us to study the fusing of protoplasts from non-hypogaea species with hypogaea lines. This approach could lead to the incorporation of beneficial genes from non commercial sources.

ACCOMPLISHMENTS IN DETAIL

Africa

Positive impacts and scientific advances through collaborative research on peanut in Nigeria has been achieved. Dr. Phindile Olorunju received her Ph.D. at the University of Georgia because of funds provided by the Peanut CRSP. Her unique expertise in virology and peanut breeding is greatly beneficial to West African programs. Peanut is an important food crop in West Africa. It constitutes a principal source of protein and dietary oil for both subsistence farmers and urban dwellers. Dr. Olorunju's work has led to a more stable production system which also provides a significant source of cash income through the sale of seed, peanut cake, dietary oil and haulms.

A significant scientific achievement was the development of a mechanical inoculation procedure to screen for resistance to groundnut rosette. A single inoculation resulted in 100% infection of plants of susceptible genotypes and about 2% of resistant plants which could be distinguished from susceptible ones on the basis of delayed time of first appearance of symptoms and disease severity. The procedure utilized highly infectious inoculum. The consistency and reliability of achieving infection makes the procedure particularly effective for inheritance of resistance studies and breeding programs.

Peanut lines are being developed and released that are adapted to the various ecological zones of West Africa (Table 1). The yield of these lines have been greatly increased. Some such as Samnut 21 (MDR-8-19) has multiple resistance to rosette, rust and leafspot. The program continues to incorporate drought tolerance and rosette resistance into lines with 90-110 day maturity.

Indeed the trend of peanut production in West Africa has been reversed. In the 1960's peanut exports were the norm. After the devastating rosette epidemic in 1975, production fell significantly below national demand. In the 1990's an increase in peanut production has been documented and in-part attributed to resistant lines having good yield attributes. The research program in West Africa, which has been supported by the Peanut CRSP, will continue to operate under national support and contribute to the national needs of their area.

Asia

Of the 26 viruses known to naturally infest peanut, PBNV, PStV, peanut clump virus (PCV), and cucumber mosaic virus (CMV) are the most important in the Asia-Pacific region. Based on research to date, the current control measures have been prescribed.

Peanut bud necrosis - Five methods using cultural practices appear to be effective. These are: adjustment of the sowing date, using high plant density, intercropping peanut with fast growing non-host crops, eradication of alternate hosts and using plastic mulch. In India it is advised to sow peanut early to avoid the thrip migratory flights so the plants are sufficiently developed to withstand infection by PBNV. Such effect has also been observed in Thailand. Peanut sown earlier in December or later in February appear to have lower PBNV incidence than those sown in mid January. Avoid planting peanut in the dry season is a good alternative. In Thailand PBNV seems to be prevalent only during the dry season.

Using high plant density is also effective in reducing losses from PBNV. This is due mainly to the compensation effect of the surviving plants. But dense plant canopy is known to reduce landing of thrips hence spreading of the disease. Canopy closure in the shortest time is less attractive to the vectors. Farmers are advised to sow good quality seed that has been treated to avoid stand thinning by pests and diseases. It was found that the incidence of PBNV in crops that were sown at low densities was proportionately higher than in those sown at high densities.

Intercropping peanut with a fast growing cereal, such as corn, millet or sorghum reduces the incidence of BND. In Thailand apart from corn, peanut is often intercropped with sugarcane which is also effective in reducing PBNV incidence. Attempts have been made to screen for peanut lines resistant to PBNV. Despite intensive efforts over many years by ICRISAT, none of the *Arachis hypogaea* genotypes tested has proved to be totally PBNV resistant. However, lines with a high level of field resistance as well as a good agronomic characteristics have been identified. Among them are ICGVs 91056,90013,90009,86598,91183,91228,86325, and 91229. Some of these lines such as ICGV 86388 performs well across India. This line also has lower PBNV incidence when tested in Thailand. Its lower infection rate when inoculated with the virus indicates its possible resistance to PBNV infection. It is expected that within a few years there should be a released cultivar that has both high yield and durable BND resistance.

Peanut stripe virus - Although PStV is detected in many weed species in peanut fields its source of primary inoculum appears to be mainly from infected peanut seed. The transmission rate of 2-5% is sufficient to lead to an epidemic of 100% incidence at harvest because of the subsequent dissemination by aphids. Therefore production and distribution of virus-free seed should be the method of choice in controlling the disease. In areas where farmers keep their own seed stocks, which is a common practice in most Asian countries, they should be advised to collect seed only from healthy-looking plants. Preparing a separate seed plot is also a good alternative, where intensive care is given to eliminate all infected plants. In some countries where government sectors play a key role in seed production and distribution, consideration should be taken in finding locations, cropping systems or seasons that are disease-free to produce seed stock. International exchanging of germplasm should take extreme precaution especially from countries where PStV is prevalent. It may be necessary to restrict the export of seed from infested areas. Plant quarantine units should develop expertise and facilities to detect PStV in seeds. At present, there is a nondestructive ELISA protocol that can be used for testing peanut seed. Like any other disease, cultivation of resistant cultivars should be the most effective method in PStV management, but resistance to PStV has not yet been found in cultivated peanut. The

screening of approximately 10,000 accessions from ICRISAT's germplasm bank, in Indonesia yielded only PStV tolerant lines. However, resistance to PStV has been identified in some wild *Arachis* species and attempts should be made to transfer this resistance to *A. hypogaea.*

Another approach is to develop peanut cultivars with very low or no seed transmission. This approach has proved to be successful in controlling PMV. This strategy would reduce both sources of primary inoculum and subsequent spread by aphid vectors. Promising genotypes have been identified in China and in Thailand. In China 7 genotypes have been screened out from over 1300 entries tested. These genotypes showed less than 1% PStV seed transmission each in three consecutive tests.

In general, insecticides have not been effective in controlling the virus vectors of non-persistently transmitted viruses such as PStV. One method of virus reduction has been the use of silver-colored plastic film. Because of its high cost, it may not be practical in routine peanut production but may have merit in virus-free seed production.

United States

Every objective of this program has largely been accomplished.

Epidemological studies have documented the increased incidence of TSWV in peanut in the southeastern United States. Two thrips vectors (*Frankliniella fusca* and *Franklinella occidentalis*) of TSWV are prevalent in the U.S. Thrips from volunteer peanuts during the dormant season were assayed for TSWV. Virus incidence in the thrips varied from 0 to 10% indicating that a possible reservoir may be overwintering thrips. Disease incidence varies from field to field and with the cultivar planted. Several cultivars have been documented to have resistance such as Georgia Browne and Southern Runner. Final incidence of TSWV at Attapulgus, Georgia in 1993 was Florunner 25.2%, Southern Runner 16.1% and Georgia Browne 13.9%. The nature of this resistance has not been determined although it does not appear to be the number of thrips resident on the peanut plants (Table 2). Yield of infected plants is greatly affected in infected early in the season (Table 3). Although several viruses infect peanut in the U.S., TSWV poses the greatest threat to production.

An efficient protocol has been developed for protoplast isolation, culture and regeneration from both cultivated and 4wild3 peanuts using a nurse culture method. Protoplasts were initiated from leaf or cotyledonary tissues and imbedded in agarose blocks and co-cultured with nurse cells of *Arachis paraguariensis.* The optimal nurse culture duration was 14 days. The optimal plating density was $2X10^4$ protoplasts per ml plating medium. Shoots developed after transfer of callus to semi-solid modified MS medium containing NAA and BA. Plantlets with normal leaflets were obtained by rooting shoots on porous rootcubes saturated with modified MS medium containing NAA. Some 4wild3 peanut species have resistance to TSWV. Preliminary studies on fusing a leaf derived protoplast from a resistant 4wild3 peanut after it was treated with x-rays to disrupt the chromosomes with a cotyledonary derived protoplast of *A. hypogaea* has been successful. This system has much promise to move genes (resistance or others of benefit) into the cultivated peanut that cannot be done by conventional breeding. Although multiple fertile plants have been obtained by this method, time did not permit full evaluation of genetic transfer.

A superior electroporation medium has been developed. An electroporation medium containing 50 mM glycine or 10 mM glycylglycine (glygly), 70 mM potassium glutamate, and 0.4 M mannitol was evaluated for its ability to improve transient $-glucuronidase (GUS) expression in immature cotyledonary protoplasts of peanuts (Table 4). GUS activity in electroporated protoplasts was 8- to 430-fold greater than that obtained using any of four other commonly employed poration media. Analysis of viability and histochemical staining of protoplasts indicated that electroporation using the glycine- or glygly-based poration medium resulted in increased protoplast viability and GUS expression when compared with other poration media. Replacement of glygly with

MES or HEPES buffers significantly reduced the level of GUS expression electroporated protoplasts. Up to 10% of the protoplasts divided and formed compact callus colonies.

The complete nucleotide sequence of the S RNA of peanut bud necrosis virus (PBNV) has been determined. It has 3057 bases, contains inverted repeats and has an ambisenese coding strategy. The sequence contains two open reading frames (ORFs), one of 1320 nucleotides located on the viral RNA (positive sense) that encodes for a Mr 49.5 kDa protein, and 831 nucleotides on the complementary strand (negative sense) that encodes for a Mr 30.6 kDa protein. ORF coding for the 30.6 kDa protein was expressed in *E. coli* utilizing pET-15b vector and immuno-blot analysis of expressed protein with polyclonal antisera against N protein confirmed that it is the N protein of PBNV. Amino acid sequence comparison of N and NSs proteins revealed identities of 22-34% with the reported tospovirus isolates of serogroups I, II, and III, whereas it had 82-86% identity with viruses in serogroup IV, watermelon silver mottle virus (WSMV) and tomato isolate of peanut bud necrosis virus (PBNV-To). From this data it is apparent that PBNV belongs to a highly diverged group that includes WSMV and PBNV-To. Two subgenomic RNA species detected in PBNV infected tissue corresponded to the predicted sizes (1.65 and 1.4 kb) of the NSs and N mRNAs. The absence of significant homology between PBNV and other tospovirus members of serogroups I, II, III and high degree of homology with WSMV and PBNV-T0 suggest that PBNV should be included in serogroup IV.

Fertile transgenic plants of peanut cv. New Mexico Valencia A were produced using an *Agrobacterium*-mediated transformation system. Leaf section explants from *in vitro* grown seedlings were inoculated with *Agrobacterium tumefaciens* strain EHA105 harboring the binary vector pB1121 containing the genes for $-glucuronidase (GUS) and neomycin phosphotransferase II (NPTII). Inoculated leaf explants were cultured on shoot regeneration medium containing kanamycin. Putative transgenic shoots were identified by screening for GUS activity and the expression of NPT II. Approximately 10% of the shoots regenerated on selection medium were GUS-positive. Five independent lines of fertile transgenic peanut plants were obtained; a frequency of 0.3% of the leaf explants inoculated. On average, 240 d were required between seed germination for explant preparation and the production of mature T_1 seed by T_0 plants. Molecular analysis of T_0 and T_1 transgenic plants confirmed the stable integration of the foreign genes into the plant genome. The GUS gene segregated in a 3:1 Mendelian ratio in the T_1 generation (Table 5).

We now have seed of transformed lines of TSWV, BNV, PCSV and PStV. Field tests to determine the degree of resistance to invasion of the infectious virus is currently being evaluated for TSWV and PStV. Studies to evaluate degrees of resistance to all transformed lines will be done by Dr. Mike Deom.

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TRAINING

A two week training course on viruses infecting peanut and other legumes was held in Pretoria, South Africa during March 1996. This training was partially supported by the Peanut CRSP. Participants were from 10 African countries. J. W. Demski was an instructor representing the Peanut CRSP.

SUMMARY

Funding for the Peanut CRSP GA/PV/N,TP has permitted numerous accomplishments:

l. World coordinated research on viruses infecting peanut was a direct result of this project. The first meeting was held at the Georgia Experiment Station in 1983. Subsequent meetings have been held in Cambridge, U.K., (ICRISAT) Hyderabad, India, Montpellier, France, and Bangkok, Thailand. Researcher on peanut viruses around the world are invited to tell of their research. Discussions are then held on what research is needed in the future and who can do the necessary studies.

2. Peanut CRSP funding supported studies that led to the first isolation and regeneration of protoplast into whole fertile plants.

3. Funding for this project permitted the development of the first successful repeatable transgenic peanut system providing fertile plants that produced seed for field testing.

4. A system of protoplast isolation and protoplast fusion with subsequent regeneration into whole fertile plants has been developed. This system with refinement can move genes from 4wild3 peanuts into cultivated peanuts. This permits gene transfer not possible by traditional breeding methods.

Table 1. Institute for Agricultural Research (Nigeria) recommended peanut varieties and characteristics.

Variety	**Maturity Days**	**Average Pod Yield**	**Shelling % kg/ha**	**Ecological Zone**	**Remarks**
SAMNUT 10 RMP 12	130-150	2800-3500	75	NGS & SGS	Rosette Resistant
SAMNUT 11 RMP 91	130-150	2800-3500	75	NGS & SGS	Rosette Resistant
SAMNUT 16 M554.76	120-130	2800-3500	74	NGS & SGS	Rosette Resistant
SAMNUT 20 M412.80I	120-130	2800-3500	73	NGS & SGS	Rosette Resistant
SAMNUT 21 MDR-8-19	120-130	2800-3500	74	NGS & SGS	Rosette, Rust & Leafspot Resistant
SAMNUT 19 K720.78	100-110	2000-2800	70	SUDAN & NGS	Rosette Tolerant
SAMNUT 18 RRB	100-110	2000-2800	72	SUDAN & PART NGS	Drought Tolerant
SAMNUT 17 48-115B	90-100	2000-2800	70	SUDAN & PART NGS	Drought Tolerant
SAMNUT 14	90-100	2000-2800	72	SUDAN SAHEL & PART NGS	Drought Tolerant

NGS - Northern Guinea Savannah.
SGS - Southern Guinea Savannah.

Table 2. Populations of tobacco thrips (*Frankliniella fusca*) and larvae of *Frankliniella* spp. on three peanut cultivars, test A5, Attapulgus, Georgia, 1993.

Frankliniella spp.	F. fusca females[a]		*F. fusca* males[a]		larvae[a,b]	
Cultivar	35 DAP	42 DAP	35 DAP	42 DAP	35 DAP	42 DAP
Southern Runner	26.1	36.1	4.7	0.8	0.3	32.3
Florunner	26.3	13.6	3.8	0.5	0.1	21.5
Georgia Browne	28.6	39.5	5.0	0.8	0.3	20.4
LSD ($P \leq 0.05$)	NS[c]	6.4	NS	NS	NS	6.9

[a] Average number of thrips per 10 termminal-quadrifolioliate leaf sampes collected 35 and 42 days after planting (DAP).
[b] Larvae were not identified to species.
[c] NS = no significant (P>0.05) cultivar effects in the analysis of variance.

Table 3. Effect of tomato spotted wilt virus infection on seed number, mean seed weight and total seed yield in individual Florunner peanut plants.

Date of Diagnosis	**Number of Seed**	**Seet wt (g/seed)**	**Yield (g)**
30 May	13.7	0.29	3.9
14 June	26.9	0.25	7.6
1 July	35.4	0.29	10.4
15 July	38.9	0.36	14.4
1 August	49.9	0.36	18.3

Table 4. Effect of potassium glutamate (KGlu) concentration on protoplast viability and GUS expression after electroporation.

GUS activity Kglu (mM)	Ionic conductivity (: mho)	Time constant (msec)	Protoplast viability (%)	(pmol MU/ min per 10^6 proptoplasts)
10	1710	119	2.2	16.5
30	2470	85	6.7	1033.3
50	3250	68	11.1	3847.9
70	3965	59	36.7	4009.3
100	5150	48	50.0	3308.6
150	6815	35	75.5	705.7
250	9940	27	71.1	451.1

Note: Fifty micrograms of DNA was used in each treatment. Protoplast viability rate and GUS activity values represent the mean of three sample observations per treatment from two experiments.

Table 5. Inheritance of GUS activity in progeny of T_0 transgenic peanut plants.

No. of T_1 T_0 plant	3:1 Segregation‡ plants tested	GUS+	GUS-	X^2	P
1-4	14	12	2	0.38	0.54
1-5	8	7	1	0.90	0.35
1-9	18	14	4	0.07	0.79
1-10	12	7	5	1.77	0.18
1-13	20	3	17	38.40	0.00
1-44	10	10	0	3.33	0.07
1-45	13	0	13	39.00	0.00
17-1	24	19	5	0.22	0.64
12-1	28	20	8	0.19	0.66
12-2	8	6	2	0.00	1.00

‡ Goodness of fit to 3:1 ratio. df=1

AAMU/FT/BF

An Interdisciplinary Approach to Optimum Food Utility of the Peanut in SAT Africa

Alabama A&M University
University of Ouagadougou, Burkina Faso
Food Research Institute, Ghana

Principal Investigators: M. Elena Castell-Perez (Alabama), Alfred S. Traore (Burkina Faso) and Kafui Kpodo (Ghana)

INTRODUCTION

Work on improved utilization of peanuts for human consumption in Semi Arid Tropical Africa (Burkina Faso and Ghana) was completed. Efforts consisted of (a) increasing utilization of peanuts in to more refined/processed forms (USA and Burkina Faso); (b) improving packaging of peanuts and peanut products to increase shelf-life (Burkina Faso); (c) utilizing peanut flour (after extraction of oil) to increase protein value of cereal-based foods (USA and Burkina Faso); (d) improving postharvest handling methods to reduce aflatoxin problem (Burkina Faso); and (e) identifying level of aflatoxin contamination at farm and processing level (Ghana).

MAJOR ACCOMPLISHMENTS

1. Weaning foods: (USA) The use of peanut paste in infant weaning foods was evaluated in terms of physical characteristics. Addition of this ingredient to mixes of cowpea, pearl millet, and teff flours decreased the water absorption index (WAI). Roasting of peanuts at 160âC for 40 minutes decreased the WAI by 25%. Research on roasting procedures was conducted to determine optimum conditions in terms of color and aroma. Qualitative and quantitative nutritional analyses of the composite flours were completed. Good formulas of weaning foods using maize, sorghum and rice, peanut flour and milk were developed in Burkina Faso. The products have improved nutritional value (18-20% protein, 10-12% fat, 2-3% minerals) and higher energy density.

2. Development of reduced-fat peanut butter: Optimum conditions (roasting, oil extraction procedures, type of fat replacers) were established for manufacture of a peanut paste with 50% less fat than commercially available products in the USA. Sensory characteristics (color, texture and taste), viscosity and storage stability (shelf- life) of product were determined.

3. Flavor and textural quality of peanut-based products: The effect of roasting and oil extraction procedures on the flavor components of peanut oil were evaluated. A peanut-butter like product was manufactured using roasted peanuts with less oil (65%). Sensory evaluation of textural and color properties of the paste were evaluated. Overall, flavor quality of the, oil was not affected by procedure and roasted defatted-peanuts showed good quality flavor composition although with less intensity (lower concentration). Sensory evaluation tests indicated overall acceptance of formulated reduced-fat peanut butter using reduced-fat peanuts and several fat replacers.

4. Development of nutritious weaning-foods using peanut paste: Four weaning blends formulated in a 60% cereal:40% legume combination of teff, pearl millet,cowpea and peanut were analyzed for differences in nutritional
quality resulting from traditional processing methods of roasting, germination and fermentation. Ingredient flours were evaluated for changes in the physical properties of particle size index, water absorption index and

water solubility index due to roasting and germination. Germination produced the most significant changes in physical properties yielding a flour with an increased particle size index, decreased dispersibility and a reduced ability to absorb water. Viscosity of cooked weaning foods made from germinated flours decreased 100 fold from blends made from unprocessed, roasted,or fermented flours. Germination improved nutritional qualities by increasing nutrient density and in vitro protein digestibility. Complementation of cereal flours with peanut paste yielded weaning foods with a significantly higher nutrient density, increased in vitro protein digestibility and lower viscosity when compared to cowpea-based blends. Sensory evaluation of the developed blends must be conducted in a country such as Ghana for determination of product acceptance.

5. Composite flour technology in manufacture of tortilla chips: Storage stability of dough for tortilla chips (masa) and rheological characteristics of fresh and frozen dough were evaluated. Dry masa flour was fortified with partially defatted peanut flour (up to 25%). Six combinations of composite flours were selected based on good dough manageability properties for tortilla chips preparation. Control dough (no supplementation) showed no cohesiveness and manageability after a few hours of preparation. The dough crumbled after frozen storage at -18oC up to 6 months. Viscosity and flow behavior changes were evaluated as a function of composition and storage. Storage conditions drastically affected the handling characteristics of the dough and all composite doughs showed improved textural properties.

6. Extruded snacks: The nutritional value, physical characteristics and shelf-life of an extruded peanut-sorghum wafer were determined. Sensory evaluation indicates overall acceptance of product.

7. Effect of fortification on dough quality of cereal-based foods (USA): Studies on proximate composition and rheological properties of peanut-fortified corn flour and dough indicate a considerable improvement on dough spreadability and shelf-life with supplementation up to 30% with defatted peanut flour.

8. Peanut paste quality and packaging (Burkina Faso): Nutritional and microbiological quality of peanut paste from several regions of Burkina Faso was analyzed. Quality of traditionally made paste was generally inferior compared to the product manufactured by a local industry. Results encourage the development of industrial processing plants for manufacture of peanut products. The optimum package size and material were identified by scientists at the Universite de Ouagadougou and information delivered to the peanut paste factory CITEC-HUILERIE.

9. Fortification and improvement of traditional or staple foods (Burkina Faso): Scientists at the University of Ouagadougou completed research on processing conditions for peanut-fortified staple foods such as "toe", "marba-tique" and "basi".

10. Studies on aflatoxin contamination of peanuts: Procedures for isolation and preparation of components for inhibition of aflatoxin production by of Aspergillus flavus with an extract of Allium sativum. Extracts of Allium sativum were very effective at suppressing the growth of Aspergillus flavus in peanuts. Furthermore, clay from various regions of Burkina Faso detoxified aflatoxins in contaminated peanut oils.

11. Identification of problems with postharvest handling of peanuts in Ghana: A survey questionnaire was improved and utilized in all regions of Ghana. Data on aflatoxin contamination and postharvest practices were collected.

IMPACTS

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 peanuts with molds, insects, and mycotoxins further limits the utility for human consumption.

Implementation of research results has led to better utilization of peanuts in Burkina Faso (weaning foods and staple foods, reduced aflatoxin contamination). Specifically, results have lead to improvement of methods of storage and packaging, processing and development of a variety of products in the country. It is expected that this knowledge will be transferred to other SAT African countries via training and/or workshops through the scientists at the Universite de Ouagadougou because the Peanut CRSP project has certainly enhanced research capability at the University of Ouagadougou to address these constraints.

Results from research conducted on the use of Allium sativum as an inhibitor of aflatoxin production on peanuts during storage will have a tremendous impact on the health conditions of the local population. The same objectives will be applied to Ghana where the conditions under which peanuts and their products are sold in the markets predispose them to rancidity development and its associated nutritional and acceptability problems, as well as microbiological and mycotoxicological implications. This emphasizes the urgent need to review and upgrade the existing processing methods and quality of traditional peanut products in Ghana.

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, the University of Ouagadougou in Burkina Faso, and the Food Research Institute in Accra, Ghana.

ORGANIZATION

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DETAILED APPROACH AND ACCOMPLISHMENTS

Comparative Study of Nutritional and Microbiological Quality of Peanut Pastes from Burkina Faso:

Nutrient composition, aflatoxin levels and microbiological contamination of peanut paste manufactured in Burkina Faso were compared. Pastes were either manufactured by the traditional method of using stone slabs or a small plate mill and then stored in containers or industrially made pastes (manufactured by CITEC-HUILERIE). The traditional pastes were collected from markets in several cities of Burkina Faso. Results indicate that proximate composition of traditional pastes varied slightly from one city to the other. Protein content (20.49-23.32%) of traditional pastes was significantly lower ($P<0.005$) than industrial pastes (30.26%). Industrial pastes were also less susceptible to rancidity (given by peroxide values 1.5-3.9 times lower than for the traditional pastes). Aflatoxin levels in traditional pastes (25.38-31.36 ppb) were considerably higher than in industrial pastes (19.13 ppb). Microbiological studies revealed noticeable contamination of traditional pastes. Results suggest that better storage, processing and handling procedures should be adopted by local manufacturers to increase product quality and shelf-life. The need for better packaging is also needed to guarantee product quality, extended shelf-life and therefore better promotion and commercialization of the paste.

MATERIALS AND METHODS

Sampling

Peanut paste samples were collected at five (5) markets from four (4) cities located in different geographical zones of peanut production: Banfora (South and Southwest), Bobo-Dioulasso (West and Northwest), Ouagadougou (Center), and Tenkodogo (East and Northeast). In each market, samples (500g) were collected from three (3) sellers in January, March and June. Samples were placed into plastic bags and taken to the laboratory for analysis. A total of 180 samples was evaluated. Market samples were manufactured using the traditional method of peeling, roasting and milling the peanut kernels using two stones. Paste is then placed into big metal containers without further protection from the environment. Samples from CITEC-HUILERIE were manufactured by following a series of steps such as cleaning, sorting, blanching, roasting and grinding.

Chemical Analyses

Moisture, free fatty acids, crude fiber, carbohydrate, fat, protein and ash content were determined using standard AOAC methods. Energy density was calculated using FAO conversions for fat, protein and carbohydrate. Aflatoxin B1 was monitored using the inoculum method. Vitamin C was determined with the 2,6-dichloroindophenol titrimetric method.

Microbiological Control

Total microflora of samples were numbered using petri plates technique with standard agar methods. Coliforms and clostridia were determined on specific media. Staphylococci, yeast and mold counts were performed using standard procedures.

RESULTS

The proximate analysis of the collected peanut paste samples is presented in Table 1. Results show that moisture content in pastes from Bobo-Dioulasso and Ouagadougou was 23-25% higher than the other pastes. This marked difference can be attributed to the fact that people add water to their product to increase the quantity (volume). Protein content of the industrial paste was considerably higher (22.9-32.3%) and carbohydrate content was lower (46.5-64.5%) than for the traditional pastes. This confirms the common use of product adulteration practices by adding sorghum or cereal flour to the product to increase product quantity.

The rancidity of pastes as indicated by the peroxide value indicate that traditional pastes became rancid more rapidly. This was expected due to the improper storage and package of the paste. Aflatoxin content of the various traditional unpacked pastes were comparable (25.38- 31.36 ppb) and much higher than for the industrial paste (19.13 ppb).

Microbiological quality of traditional pastes was generally poor (Table 2). In contrast, the level of microbial contamination in the industrial paste was negligible. These results point out the high health risk at which consumers are exposed by purchasing these products.In summary, the research showed that industrial processing of peanut paste yields a product with good nutritional quality and free of contamination by microbes and/or aflatoxins. Therefore, local traditional peanut paste manufacturers must be educated on practices to improve their products.

Table 1.

Proximate Composition of Peanut Pastes Sampled in Different Cities of Burkina Faso

	Mean values of parameters on peanut pastes at various locations1				
Parameter	B.a	B.-D.b Ouagac	Tenk.d	CITEC-HUILERIEe	
Moisture (%)		0.77	1.08	1.06	0.86	0.80
Free fatty acids (%)		0.60	0.69	0.54	0.30	0.57
Fat (%)		51.26	49.30	49.15	50.29	51.60
Protein (%)		20.63	23.32	22.04	21.48	30.26
Carb. (%)		25.06	22.31	24.48	23.26	15.23
Fiber (%)		1.80	1.44	2.33	2.50	2.20
Ash (%)		2.59	2.46	2.47	2.47	2.55
Heat content (kcal/g)		6.33	5.85	6.02	5.66	6.11
Vitamin C (mg/100g)		14.00	14.80	13.07	13.86	13.71
Peroxide value (mMO2/kgoil)		11.93	16.75	35.80	18.03	9.08
Aflatoxin B1 (ppb)		31.36	30.31	26.33	25.33	19.13

1 Mean values from 45 samples
a Banfora, b Bobo-Dioulasso, c Ouagadougou, d Tenkodogo
e Mean values from 15 samples

Table 2.

Microbial Contamination in Peanut Paste Samples from Different Cities of Burkina Faso

	Mean values of microbial counts on peanut pastes at various locations1				
Microorganism	B.[a]	B.-D.[b]	Ouagac Tenk.[d]	CITEC- HUILERIE[e]	
Total micro-flora (.103/g)	1529.0	1130.0	1239.0	1896.0	0.00
Coliforms (.103/g)	1.1	48.9	2.2	21.1	0.00
Staphylococci (.103/g)	114.5	82.9	672.6	616.6	0.00
Salmonella/ Shigela (.103/g)	0.7	2.6	0.6	16.1	0.00
Clostridia (.103/g)	53.7	169.9	280.2	128.2	0.00
Yeast/mold (.103/g)	268.5	79.5	12.1	80.5	0.00

1 Mean values from 45 samples
a Banfora, b Bobo-Dioulasso, c Ouagadougou, d Tenkodogo
e Mean values from 15 sample

Dough Quality:

Storage of dough presents a big challenge in the food industry because the stability of frozen dough during storage, and during freezing-thawing remains a problem. The purpose of this research was to analyze the effect of peanut supplementation on corn (masa) flour storage quality. Studies on rheological changes of dough due to temperature and composition are currently in progress.

MATERIALS AND METHODS

Dry masa flour (DMF) was fortified with chickpea flour (CPF) (up to 30%) and partially defatted peanut flour (PDPF) (up to 25%). Six combinations of composite flours were selected based on good dough manageability properties for processing. This was determined by handling and sheeting the fresh dough, the frozen-thawed dough and by making a tortilla-like product. The masa and composite dough were stored at -18âC for 6 months and defrosted for analysis. Water activity of the dough during storage was monitored using a Rotronic measuring system. Textural characteristics (compression force) were determined using an Instron Universal Testing Machine for fresh and stored samples (every two weeks for up to 6 months). Proximate analysis of samples was done using standard procedures.

RESULTS

Fortification of dry masa flour improved the textural characteristics of the dough during frozen storage (lower values of compression force which indicate a softer product versus the hard texture of masa dough). Dough manageability (cohesiveness) also improved with fortification. Masa dough lost plasticity during storage due to greater water loss as compared to the composite dough which remained acceptable for processing. Proximate analysis of flours showed a desirable increase in protein content (8 to 16%). However, fortification also increased fat (3-8%) and moisture content (8- 14%, wb.). Results indicate that fortification of dry masa flour produced a dough with acceptable quality (textural) after 6 months of frozen storage.

Field Survey on Peanut Production in Ghana:

The objective of the survey was to study peanut production in the major peanut growing areas of Ghana. The survey covered the Ashanti, Brong-Ahafo, Northern, Upper-East, and Upper-West regions. Information obtained from the survey included the general background of peanut farmers, practices for land preparation, planting, harvesting, threshing (stripping), drying, shelling, storage, and general problems which hinder peanut production in Ghana. A summary of results from fifty farmers is given below:

Peanut is grown by small-scale farmers with an average planted area of 3.5 acres. Two thirds of the farmers had not had any formal education. Severeal varieties are planted with the most widely cultivated ones preferred because they are easy to harvest (by manual pulling from the soil), early maturing and high yielding. Except for land preparation which was carried out by both manual means and in some cases by use of tractors, all other operations such as planting, harvesting, threshing, and sorting were manually done.

Drying of peanuts after harvesting was carried out directly in the sun either on the farm or at home. Most farmers store peanuts unshelled in jute sacks or in traditional storage structures until they are ready to sell. Storage could be for as long as 9 months. Only 8% of farmers interviewed used shellers. 35% of farmers did not sort peanuts and those who sorted did so to get a good market price for their produce. All the farmers interviewed were completely ignorant about the aflatoxin problem in peanuts.

Buyers of peanuts at the farmgate are local traders and middlemen from the urban centers. Major problems mentioned by peanut farmers were unreliable rainfall, poor soil fertility, and lack of farming inputs for land preparation, harvesting, threshing, shelling, and high labor cost. All the farmers interviewed were willing to adopt new equipment for peanut production if offered to them on a hire-purchases basis.

Studies on Existing Traditional Processing Procedures for Peanut Butter in Ghana:

A nationwide survey covering the ten regions of Ghana was conducted to study the existing traditional methods for the preparation of peanut butter. The major unit operations identified were roasting, dehulling, winnowing, milling, and packaging. (See attached: a complete survey report).

Quality Evaluation of Traditional Peanut Butter Samples in Ghana:

Twenty peanut butter samples from the Eastern and Central regions of Ghana were analyzed for aflatoxin contamination (B1, B2, G1 and G2) using High performance Liquid Chromatography (HPLC). Generally, extremely high levels of aflatoxin were detected in the samples. Only two out of the twenty samples were aflatoxin-free (detection limits for aflatoxin B1 and B2 of 0.04 :g/kg and for G1 and G2 of 0.06 :g/kg). All the positive samples had levels above the WHO/FAO/UNICEF maximum permissible limit of 30 :g/kg for protein supplements. Total aflatoxin levels for positive samples from the Eastern region (Table 3) ranged from 47.7 to

2024.1 :g/kg. Total aflatoxin level for the Central region (Table 4) was 78.0 to 2013.9 :g/kg. Regions in the Northern part of the country, namely Upper West, Northern, and Upper East regions had low aflatoxin levels relative to other regions. Generally, all four types of aflatoxin were detected in all regions suggesting contamination by both Aspergillus flavus and Aspergillus parasiticus. In the case of the Central region, however, aflatoxin G1 and G2 were less frequently detected suggesting a dominance of Aspergillus flavus (responsible for the production of aflatoxin B1 and B2) over Aspergillus parasiticus. Range and mean of total aflatoxin values in the ten regions are listed in Table 3 below. Ten samples of peanut butter from the Eastern region were analyzed for moisture, protein, fat, and free fatty acids. Moisture levels ranged from 0.7% to 3.0%. This may be due to differences in the extent of roasting. Protein and fat contents were characteristically high ranging from 27% to 36% and 40% to 53%, respectively. All but one peanut butter samples had free fatty acid (FFA) values within the range of 0.5% to 1.3% (as oleic). One sample had a FFA content of 5.8% which was extremely high. Most oils produce noticeable rancidity to the palate when the FFA is about 0.5-1.5%.

The microbiological quality of twenty five samples from the Eastern, Central and Western regions was evaluated. Microorganisms isolated included Bacillus spp, Candida sp, Aspergillus niger, A. ochraceus, A. candidus, A. flavus, and A. parasiticus. Other molds isolated in smaller numbers were Penicillium spp, Torula sp., and Monilia sp.. However, Aspergillus spp. were found to be the most prevalent and accounted for about 80% of the total mold population of the samples. Bacterial count of the samples ranged from 120 to 9.3x105 CFU while mold count ranged from <10 CFU to 5.6x105 CFU. No pathogenic bacterial organisms (E. coli, Staphylococcus aureus and Salmonella spp.) were isolated.

Table 3.
Aflatoxin Content of Peanut Butter Samples From Various Regions of Ghana

Region	**Range for Positive Samples (ug/kg)**	**Mean Aflatoxin Levels (ug/kg.)***
Eastern	48 - 2024	663 (10)
Western	14 - 3278	727 (9)
Central	78 - 2014	531 (10)
Upper-East	10 - 285	53 (10)
Northern	2 - 205	55 (10)
Upper-West	2 - 52	9 (11)
Brong-Ahafo	4 - 358	88 (10)
Ashanti	7 - 3057	632 (8)
Volta	98 - 1856	710 (10)
Greater-Accra	16 - 436	133 (12)

* Levels based on total number of samples analysed.
( ) Number of samples analysed in the region.

PUBLICATIONS AND PRESENTATIONS

Manuscripts

Castell-Perez, M.E. and Mishra, A. 1995. Flow behavior of regular and peanut- fortified idli batters. J. of Texture Studies 26(3): 273-279.

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Nikiema, P.A. and Traore, A.S. 1995. Etude de la contamination des graines d'arachide par les aflatoxines produites au cours du stockage. J. Africain de Recherces Chimiques 1(1): 2-14.

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Pan, B. and Castell-Perez, M.E. 1997. Textural and viscoelastic characteristics of canned biscuit dough during microwave and conventional baking. Journal of Food Process Engineering. In press.

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Presentations

Duarte, N. and Castell-Perez, M.E. 1995. Changes in texture of masa dough due to storage and composition. Institute of Food Technologists, Anaheim, June 3-7.

Pan, B. and Castell-Perez, M.E. 1995. Effect of peanut supplementation on texture of wheat dough for microwave baking. Institute of Food Technologists, Anaheim, June 3-7.

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Duarte, N. and Castell-Perez, M.E. 1996. Effect of frozen storage and composition on the viscosity of masa. IFT International Meeting, New Orleans.

Singh, S.K. and Castell-Perez, M.E. 1996. Characterizing the rheological behavior of reduced-fat peanut paste. IFT International Meeting, New Orleans.

Torres, H. and Castell-Perez, M.E. 1996. Rheology and shelf-life of reduced-fat peanut butter. Research and technology Transfer Symposium, Texas A&M University.

THESIS AND DISSERTATIONS

Mr. Bin Pan - Thesis Topic: Effect of peanut flour supplementation on textural and rheological changes of wheat dough during microwave baking. M.S. Degree, July 1994.

Mr. Iwan Surjawan - Thesis Topic: Physical and functional properties of a sorghum-peanut extruded wafer product. M.S. Degree, December 1994.

Ms. Wichayada Intaratip - Thesis Topic: Nutritional assessment of sorghum and peanut-based wafers for military rations. M.S. Degree, May 1995.

Mr. Brent Jones - Thesis Topic: Storage stability of sorghum-peanut wafers. M.S. Degree, June 1995.

Mr. Rufin Simde (Burkina Faso) - Thesis Topic: Improvement of shelf-life of peanut products (roasted peanut kernels and locally made peanut pastes). Doctorat Degree (December 1994).

Mr. Simeon Nanema - Thesis Topic: Improvement of nutritional quality of toe on fortification with peanut proteins. Doctorate Degree, December 1995.

Ms. NellyDuarte. Rheology of Composite Dough: Processing and Storage Quality. PhD Dissertation. December 1996.

Ms. Linda Griffith. Physical and Nutritional properties of Selected Weaning Blends. PhD Dissertation. July 1996.

Mr. Surendra K. Singh. Rheological and Flavor Properties of Reduced-Fat Peanut Paste. M.S. Thesis. May 1996.

International Travel

April 1995. Dr. Alfred Traore, principal Investigator from Burkina Faso, and Ms. Kafui Kpodo, Principal Investigator from Ghana, visited Alabama A&M University to discuss research protocols and to revise project objectives. They previously attended the Peanut CRSP Workshop in Arlington, VA, March 29-31.

Two round trip tickets to Ghana were purchased for Dr. Okezie and Dr. Nwosu to visit Ghana and attend the 5th Regional Groundnut Workshop for West and Central Africa, as well as to discuss implementation of the new phase of the project.

Progress made Toward Resolving Constraints

1. Socioeconomic constraints:

- Insufficient local food supply or inadequate food technology prevents growers, processors, or consumers from exploiting the full potential of peanut because it is not considered as a primary food source.
- Information not available to potential beneficiaries of new peanut production and use technologies.

Through the Peanut CRSP Food Technology project information on technology, packaging and development of new peanut-based products has been presented to local peanut processors in Burkina Faso. Packaging of peanut-based products and consumption of peanut-fortified weaning foods is currently a common practice in that country. More people are directly dealing with peanut products which creates an increase in the activity of the informal sector.

2. Health and nutrition constraints:

- Health hazard from mycotoxin in contaminated peanut.
- Methods needed to incorporate peanut and peanut products into safe and nutritious processed foods.

People have been informed of the importance of hygienic practices during handling and storage of peanuts and peanut products. Alternative methods for prevention of mycotoxin contamination of peanut-based products have been developed and should be put into practice soon. A direct relationship between mycotoxins and liver cancer was established and proper organisms were informed. The nutritional value of staple foods was enhanced by incorporation of peanut flour. Some products are currently in preparation for marketing studies. A survey to identify the levels of contamination in Accra, Ghana, has helped local scientists to establish the application of methodologies to decrease or eliminate the contamination problem.

3. Research capacity and tools:

- Inadequate number of trained research and support personnel in host countries.

The project has furnished equipment to the Food Science Laboratory at the University of Ouagadougou in Burkina Faso and the Food Research Institute in Ghana. Several students have been funded by the project and some are actively working with peanut research problems in Burkina Faso. Field technicians were hired in Ghana for survey work.

GA/FT/TP

Appropriate Technology For Storage/Utilization of Peanut
University of Georgia - Thailand and Philippines

Larry R. Beuchat, Principal Investigator, UGA
Penkwan Chompreeda, Principal Investigator, Thailand
Virgilio V. Garcia, Principal Investigator, Philippines

INTRODUCTION

Efforts in FY96 were focused on transferring processes to utilize peanuts and peanut products in traditional and new food products which would be acceptable in Thailand, the Philippines, other Southeast Asian countries and the U. S., and on a Training Course on Peanut Utilization in Thailand and an Aflatoxin Workshop in The Philippines. The choice of products for technology transfer was based in large part on consumer survey response but also on intuition and insight to consumer behavior. Fundamental and applied research was conducted in laboratories of all three collaborating institutions. Utilization of peanut and peanut products in a wide variety of food products has resulted in the development of several items which we believe are highly acceptable and have excellent potential for marketing.

A workshop on training trainers on "Quality Evaluation and Utilization of Food Legumes" was held at Kasetsart University and a Seminar-Workshop on "Aflatoxin Control for the Food Industry" was held at the University of the Philippines at Los Baños. Both events were well attended and represent a transfer of knowledge and information gained by Peanut CRSP researchers over the life of the project.

MAJOR ACCOMPLISHMENTS

A. Kasetsart University (KU) (Host Country)

A peanut-based cereal bar formulation was optimized to achieve high nutritional value (10% RDA for adult) and consumer acceptability.

Products containing fermented peanut (peanut tempeh) were developed and tested for consumer acceptability in Nongno Village, Kud-Jub District, Udonthanee Province.

A mosquito repellant product made from peanut hulls and other natural materials, including bergamot, was successfully developed and tested.

A training workshop on "Quality Evaluation and Utilization of Food Legumes" was held April 22 to May 24, 1996. The workshop was attended by 13 participants from 6 countries in Asia and Africa.

B. University of the Philippines at Los Baños (UPLB)

Modification of formulations of peanut products developed earlier in the project were done for the purpose of enhancing quality and acceptability.

A 2-day workshop on "Peanut Processing was held at the Institute of Food Science and Technology (IFST).

A 2-day seminar-workshop on "Aflatoxin Control for the Food Industry" was also held. Representatives from food industry, government and academia attended.

C. Visayas State College of Agriculture (ViSCA)

Issues of training, economic benefits market distribution schemes, material sourcing and packaging technologies associated with peanut product processing by womens cooperative groups were evaluated.

Enhancement of quality of two peanut products (Lady Button and Lady Finger) was achieved using a response surface methodology.

The project expanded the number of adopters by introducing peanut-enriched products to the Bubon Integrated Root Crop Processing Cooperative, the San Agustin Rural Improvement Club and a private entrepreneur.

D. University of Georgia (UGA)

A honey-peanut butter formulation was developed and optimized for consumer acceptability.

Peanut film production methods were evaluated. Qualities of films produced by surface film and deposition methods were assessed.

The kinetics of drying peanuts in terms of energy cost were studied. A simulation program (PNUTDRY) is valid in predicting drying conditions and can be used in the design of peanut dryers of desired capacity with optimum drying conditions.

Work to identify biochemical the mechanism(s) by which a bacterium (*Flavobacterium aurantiacum)* degrades aflatoxin continued. Calcium and magnesium enhanced the removal of aflatoxin.

IMPACTS

The impact of technology transfer to enhance the well-being of small farmers in the Huay-Bong-Nua and Kud-Jub village areas in Thailand continues to be evident. The initiation of women cooperator groups who will process and market peanut products in two rural areas in Leyte, Philippines has had a positive impact on the families involved in this project.

A workshop on "Quality Evaluation and Utilization of Food Legumes", conducted by project members at Kasetsart University, has increased the level of awareness and knowledge of a wide range of peanut utilization topics on an international scale. Likewise, the workshop on "Aflatoxin Control for the Food Industry," conducted by project members at the University of the Philippines at Los Baños, will have an extremely important and long-lasting impact on the awareness of safety of peanut and peanut products in the Philippines. The success achieved by these workshops is strong evidence of the knowledge and organizational abilities attained, in part, by involvement of researchers in Peanut CRSP during the preceding years of the project. The impact of contributions of team members will continue long after the project is terminated.

GOALS

A goal of the project is to enhance the capabilities of scientists, technicians and students at KU, UPLB, ViSCA and UGA. In time, these scientists will assist in disseminating information on peanut utilization in its broadest sense to research scientists, teachers, government and industry personnel and laypersons on a global scale but particularly in Southeast Asia. The education and training made possible through the project will enhance

possibilities to improve the nutritional well-being of the consumer. At the same time, the economic well-being of the peanut farmer, the peanut processor and the peanut marketer will also be improved.

OBJECTIVES (1995-96)

A. Kasetsart University

1. Continue to develop a breakfast bar using undersized peanut as a source of protein. Shelf-life and consumer acceptance of this product will be evaluated.

2. Continue to implement the utilization of peanut tempeh in food by housewives at village level.

3. Continue to utilize peanut hulls in development of a mosquito repelling stick.

4. Cooperative with the Department of Agriculture Extension, Ministry of Agriculture to transfer technology of peanut processing to home economists and industrials in Northern and Northeastern Thailand areas.

5. Cooperative with the Department of Technical and Economic Cooperation, Office of Prime Minister to offer a training course on quality evaluation and utilization of legumes to train participants from Indo-China countries and other countries under Thai-AID projects.

B. University of The Philippines at Los Baños

1. Characterization of the compound(s) produced *Cladosporium fulvum* inhibit the growth of aflatoxigenic aspergilli.

2. Technical assistance will be given to small-scale peanut handling and processing industries for the purpose of improving quality and safety (aflatoxin contamination) of peanut products.

C. Visayas State College of Agriculture

1. Work on peanut-based snacks and confectionary products.

2. Develop and evaluate equipment and tools for small-scale drying of peanuts and for processing peanut-based products at women's cooperatives.

3. Transfer technologies to increase utilization of peanuts in value-added products to small- and medium-sized cooperatives operated and managed by women.

D. University of Georgia

1. Work will be done to develop an acceptable model for characterizing flavor changes in full-fat and defatted roasted peanuts during storage by correlating 4-6 volatile compounds with subjective sensory scores.

2. Initiate research on the flavor enhancing properties and nutritional contribution of peanut meal/flour and fermented meal/flour in food systems.

3. Investigate the suitability of using edible films on controlling oxidation of roasted, stored peanuts.

4. Determine, chemical and sensory properties of a beverage prepared from roasted peanuts using various processing technologies.

5. Formulate snack products containing flours prepared from partially defatted peanuts and cowpeas subjected to solid-state fungal fermentation.

6. Study mechanical properties of selected peanut cultivars as related to handling, storage and processing.

7. Develop an interactive computer model to design peanut drying systems.

ORGANIZATION AND PERSONNEL

A. <u>Kasetsart University (KU)</u>

<u>Department of Product Development</u>

Dr. Penkwan Chompreeda, Principal Investigator
Mr. Vichai Haruthaithanasan, Co-Investigator
Dr. Chintana Oupadissakoon, Co-Investigator
Sr. Paisan Wuthijumnong, Co-Investigator
Mr. Sombat Khotaveewattana, Co-Investigator
Dr. Haraivat Uaphithak, Co-Investigator
Ms. Wimolsiri Thanavisutha, Graduate Student (M.S.)
Ms. Supang Reungchay, Graduate Student (M.S.)
Mr. Panuwat Suppakul, Graduate Student (M.S.)
Ms. Nantana Chutimantanon, Laboratory Technician
Ms. Sathit Litlerchai, Research Technician (assisting, no support)
Ms. Kasina Boonsaeng, Research Technician (assisting, no support)
Ms. Chantana Kopayakkin, Research Technician (assisting, no support)
Ms. Wasana Chaijaroon, Typist (assisting, no support)
Ms. Bunkerd Walkreu, Secretary (assisting, no support)

B. <u>University of the Philippines - Los Baños (UPLB)</u>

<u>Institute of Food Science and Technology</u>

Dr. Virgilio V. Garcia, Principal Investigator
Dr. Richardo R. del Rosario, Co-Investigator
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Roberta Lauzon, Graduate Student (partial support)

C. Visayas State College of Agriculture (ViSCA)

Department of Agricultural Chemistry and Food Science

Dr. Lutgarda S. Palomar, Principal Investigator
Dr. Lemuel M. Diamante, Co-Investigator
Dr. Julieta R. Roa, Co-Investigator (Socio-Economics)
Dr. Christina R. Arcena, Co-Investigator (statistics)

D. University of Georgia

Center of Food Safety and Quality Enhancement

Department of Food Science and Technology

Dr. Larry R. Beuchat, Principal Investigator
Dr. Manjeet S. Chinnan, Co-Investigator
Dr. Robert E. Brackett, Co-Investigator
Dr. Anna V. A. Resurreccion, Co-Investigator
Dr. P. Mallikarjunan, Post-doctoral Associate (partial support)
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Mr. Witoon Prinyawiwatkul, Thai Graduate Student (M.S.)
Mr. Anuvat Jangchud, Thai Graduate Student (Ph.D.)
Mr. Agustin Ramos, Filipino Graduate Student (M.S.)
Ms. Doris D'Souza, Graduate Student (Ph.D.)
Mr. Zhong Zhang, Graduate Student (M.S.) (partial support)
Ms. Barbara Adler, Research Technician II (partial support)
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Ms. Brenda Nail, Agricultural Research Coordinator I (assisting, no support)
Mr. Glen D. Farrell, Research Technician III (assisting, no support)
Mr. Lary Hitchcock, Research Technician III (assisting, no support)
Ms. Julia Heggie, Laboratory Research Technician II (assisting, no support)
Ms. Kimberly Hortz, Research Technician I (assisting, no support)
Ms. Sandra O'Pry, Research Technician I (assisting, no support)
Ms. Ann Autry, Secretary (assisting, no support)
Ms. Alice Neal, Secretary (assisting, no support)
Ms. Rebecca Uber, Accounting Assistant (assisting, no support)

Ms. Shirley Wade, Secretary (assisting, no support)
Ms. Brenda Banister, Accounting Assistant (assisting, no support)

ACCOMPLISHMENTS IN DETAIL

RESEARCH

A. Accomplishments at Kasetsart University (KU)

1. Development of a Ready-to-Eat Mixed Cereal Bar

This study was conducted to develop a nutritious ready-to-eat mixed cereal bar using Thai agricultural products. A consumer survey was conducted to identify a desirable prototype product of ready-to-eat mixed cereal bar. Results from 100 consumers indicated that they preferred a bar in rectangular shape with a serving size of 50-100 g. Consumers preferred a product containing pineapple flavor, and that was crispy in texture and moderately sweet. The product was formulated to meet 10% Thai RDA for Thai adults. Linear programming was employed to formulate a bar to meet protein, iron, vitamin A, thiamin, riboflavin and niacin requirements. Six formula bars were prepared and quality characteristics were measured. Statistical analysis were employed to optimize the bars.

The optimum formula contained 15% roasted glutinous rice, 20% roasted peanut, 18% roasted sunflower seed, 3.2% maltose syrup, 15% pineapple jam, 5% baked raw banana, 5% each of skim milk powder, jaggery and water, 4.3% honey, 0.5% each pineapple flavor and salt. The process for preparing the product is shown in the Fig. 1. The product is light brown in color, and has a compression force of 62.8 Newton and a_w 0.37. The product contained 5.96% moisture, 25.21% fat, 1.80% ash, 12.50% protein 38.11% crude fiber and 60.40% carbohydrate. One serving size (60 g) contained: energy, 423.6 Kcal; vitamin A, 72.11; vitamin B1, 0.15 mg; niacin, 0.13 mg; and iron, 2.11 mg. The product was microbiologically safe. An accelerated storage study revealed that the shelf-life of the product packed in aluminum bags is 73 days at 30°C. Acceptability tests indicated that consumers moderately accepted the ready-to-eat mixed cereal bar.

```
Skim milk powder          Pineapple jam          Roasted glutinous rice,
      *                   maltose syrup,         Roasted peanut
      *                   honey, jaggery, salt   Roasted sunflower,
      *                         *                Baked raw banana
      *                         *                      *
Add water and mix              Mix                   Mix
      *                         *                      *
      *                         *                      *
                *                                    *
        Heat (final TSS = 88)                        *
                *                                    *
                              Mix
                               *
                          Weigh 20 g each
                               *
                   Mold and Press at 100 psi 30 sec.
                               *
                          Dry at 70°C 20 min.
                               *
                   Package in aluminum foil bag
```

Fig. 1. Flow diagram for preparing ready-to-eat cereal bar.

2. Production of Tempeh Starter in a Cottage Industrial Scale

Peanut tempeh has potential as a protein source for Thai consumers. Several products prepared from peanut tempeh and introduced to consumers by our project team were acceptable and promising. Production of tempeh starter was done on a cottage scale. Substrate was prepared by mixing rice flour, rice bran and water in ratio of 9:1:7, pressing the mixture (0.5 cm. thick) sterilizing at 121°C for 15 minutes, cooling, dipping in a *Rhizopus oligosporus* spore suspension, incubating at room temperature for 65 hours, grinding and packaging. Color values of the starter were: L = 63.9 a = 0.1 b = 10.7; water activity was 0.79. Initial spore population was 8 x 10^5 CFU/gram. Efficiency of the starter for producing mycelium in peanut tempeh was 18-23 hours at 25-32°C. Results of storage tests indicated that color and water activity of the starter were reduced at the end of storage period (12 weeks). Utilization of the starter by farmer's housewives at Nongno Village and Kud-Jub District, Udonthanee indicated that tempeh had qualities similar to those of tempeh prepared in the laboratory. Housewife groups used peanut tempeh in food products; Jaewbong, Namya, Pudprikkhing groups accepted products very well. This study will continue as part of technology transfer project.

3. Development of Mosquito Repelling Sticks Using Peanut Hulls

This study was conducted to develop a mosquito repellant coil from natural materials and peanut hulls. Results from a consumer survey indicated the product should be in the form of a coiled contain bergamot aroma and have a green color. Quality evaluation of commercial mosquito coils (5 brands) revealed that they met the standard requirement of TISI. In preparing ingredients, the yield of ground peanut hulls, citronella, bergamot leaves and tangerine peels was 74.9%, 43.3%, 40.3% and 20.6%, respectively. A mixture design was employed in product formulation. The optimum formula consisted of ground peanut hulls (39.2%), citronella (14.8%), bergamot leaves (9.8%), tangerine peels (4.9%), pregelatinized tapioca starch (14.8%), Bong (14.8%), green color (0.2%), bergamot flavor (1.0%) and sodium benzoate (0.5%). The process for preparing the product is shown in the Fig. 2. The product is light green and weighs 12.4 g/coil. It has a bulk density of 0.57 g/cc, a burning rate 36.6 min/g, a hardness of 24 N and a water activity of 0.55. It contains 10.2% moisture, 4.2% fat, 3.8% ash and 42.8% crude fiber. Repellant efficiency of this product was 80% for up to 30 min. When the coil is packed in an envelope inside a carton and stored at 65% RH it remained stable for 100 days. Consumers liked this product moderately to very much.

Dry ingredients (ground peanut hulls,
citronella, bergamot leaves, tangerine peels
pregelatinized tapioca starch, Bong, green
color, bergamot flavor and sodium benzoate)
(100 part)
*
Add water (95 part)
*
Mix
*
Form into stick (85 cm length, diameter 0.5 cm)
*
Coil
*
Dry at 60°C 80 min
*
Cool
*
Package in paper bag

Fig. 2. Flow diagram for preparing mosquito repelling sticks.

B. Accomplishments at the University of the Philippines at Los Baños (UPLB)

The FY96 budget for the University of the Philippines at Los Baños did not permit extensive research activities. Funds were spent largely on conducting a Seminar/Workshop on Aflatoxin Control for the Food Industry (see TRAINING ACCOMPLISHMENTS). Summarized here are research activities that were initiated in years previous to FY96 but received additional research and outreach attention in FY96.

1. Development and Improvement of Peanut Products

a. Development of Peanut Soft Curd

A study was undertaken to utilize peanuts/peanut milk for the development of a soft curd to be used as a snack item for school children. Soft curd was developed from peanut milk prepared from steam-blanched and water-blanched peanuts. Peanut milk supplemented with 0.5% $CaSO_4$ or $MgCl_2$ and agar-agar (0.3-0.4%) produced a soft curd with good consistency and texture. The use of agar-agar as a firming agent together with sucrose (12 - 18° Brix) produced a palatable soft curd comparable to soft soybean curd. Soft peanut curd prepared using 0.5% $CaC1_2$ was not acceptable because of a bitter after taste. Proximate analysis showed that peanut soft (wet weight basis) curd contained low amounts of protein (0.8 - 1.4%) and fat (0.6 - 0.8%) which were similar to commercial soft soybean curd. Strength of the soft peanut curd ranged from 2.5 - 5.2 x 10^2 dyne/cm^2 compared to 1.5 x 10^2 dynes/cm^2 for soft soybean curd. Soft peanut curd made from slightly roasted (60 - 65°C) peanuts had higher lightness (L) values than curds made from steam-blanched or water-blanched peanuts. Soft peanut curd stored at 4°C for 7 days did not undergo microbial spoilage while curd stored at room temperature (30 - 32°C) underwent spoilage within 1 day. The acceptability of soft peanut curd was further tested using 72 Grade IV students from Maquiling School, Los Baños, using a 7-point Hedonic scale (Smiley). Results showed that soft peanut curd was acceptable to the students with an average score of 2.48 (1 = highly acceptable; 7 = not acceptable).

b. Studies on Fermented Peanut Milk

i. *Yogurt*

Studies were done to determine the suitability of peanut milk as a substrate for bacterial fermentation. The production of peanut milk was optimized. Five lactic acid bacteria (*Lactobacillus acidophilus, L. bulgaricus, L. helveticus, Streptococcus diacetylactis* and *S. thermophilus*) were tested for the production of fermented peanut milk. All test strains except *L. bulgaricus* produced acid in sufficient amount to reduce pH and cause coagulation of peanut milk. Approximately 0.11% titratable acidity was needed to cause coagulation. Coagulation occurred at about pH 5.0 and after 4 - 8 hours of incubation. Fermented blanched peanut beverage had a firm yogurt consistency with syneresis occurring after 8 h fermentation. Peanut yogurt prepared using *S. diacetylactis* had a more desirable aroma than yogurts prepared using the other test cultures. While other yogurts retained the raw peanut aroma and flavor, yogurt made using *S. diacetylactis* lacked this aroma and flavor. Generally, after 24 h of fermentation, an acid aroma masked the undesirable strong peanut aroma in all fermented peanut yogurts. A ratio of 1 part peanut kernels to 4 parts water yielded a product containing 10% total solids and 90% moisture.

Studies were done to improve the texture using stabilizers. Carageenan and sodium alginate were tested in the preparation of yogurt from peanut milk. Carageenan-enhanced product appeared to be better than sodium alginate-enhanced ones. The curd was firmer and more uniform. The use of sucrose and glucose as energy sources were also investigated. Both sugars enhanced the decrease in pH. Addition of glucose gave a more desirable product. The product sweetened with sucrose had undesirable aroma and off-odor.

ii. *Yogurt drink*

Yogurt drink was prepared from a combination of peanut milk and reconstituted peanut milk (RSM) because of the limitations of the full-yogurt type product from peanut milk. *L. bulgaricus* and *S. thermophilus* were added at 1.5% each. RSM at 8% and 10% levels were added to the peanut milk at different substitution levels. The

milk was then incubated at 37°C for 24 h. Percent titratable acidity, pH, total soluble solids and organoleptic quality were analyzed. Preliminary results showed that RSM at 8% was unsuitable as substitute for yogurt drink preparation. The product using 8% RSM and peanut milk has a characteristic chalkiness and unpleasant taste. RSM at 10% was found suitable for substitution.

c. Peanut Majareal Improvement

Two established recipes for peanut majareal preparation (IFST peanut bar and DOST dulce majareal) were followed. Each were subjected to proximate analysis (AOAC Standard Methods of Analysis). Sensory evaluation showed significant differences in color, texture, fracture and adhesiveness but no significant difference in peanut flavor and a comparably high acceptability.

Microbiological tests were done to determine the shelf life of peanut majareal stored at room (30°C) and refrigerated (10°C) temperatures. Plating was done using acidified potato dextrose agar (PDA), malt yeast agar (MYA) and nutrient agar (NA) to determine mold, yeast and bacterial counts respectively. Sampling was done on a weekly interval until microbial growth became visible. For the product stored at room temperature, mold growth was observed on the surface on the 28th day of storage. Growth was not observed on samples stored at refrigerated temperature even after two months of storage, but plating was terminated on the 35th day. The use of different antimicrobial agents was also studied. Sodium benzoate, potassium sorbate and sodium propionate were added to peanut majareal at 0.1% level (w/w) and stored at room temperature, and mold count was determined every week. All the antimicrobial agents were able to inhibit mold growth up to 49th day of storage but sodium propionate showed maximum inhibition as indicated by the very low microbial count on the final day of analysis.

Peanut majareal develops rancid flavor during storage, so the use of an antioxidant, butylated hydroxy anisole (BHA) at 0.02% of its fat content was studied. The product was then stored at room and refrigerated temperatures and analyzed for free fatty acids on a weekly interval. Results showed that addition of BHA can stabilize the fats present in the product and thus may delay or inhibit the onset of rancidity at room and refrigerated temperature storage.

d. Soft White Cheese

Soft white cheese was developed from peanut using coagulants. Rennet, acetic acid and the combination of the two coagulants were tested. Only acetic acid was used for the production of soft white cheese from peanut milk (PM). PM at 25 - 100% was combined with cow's milk and used for soft cheese production. Results showed that the most acceptable level of PM substitution in soft cheese making was 25% peanut milk.

e. Peanut Sauce

Three peanut sauce formulations were developed. Sensory evaluation indicated high acceptability scores. Bottled sauces with pH adjusted to 4.5 were also subjected to sensory evaluation and had moderate acceptability.

f. Peanut Cakes and Candies

Studies on peanut product development involves the development of peanut cakes and candies. Peanut kisses, a sweet confectionery, was prepared from defatted and roasted peanut meal. The oil (21.4%) had been extracted from the peanut to produce the meal (76%). The peanut kisses turned brown on baking. A lower temperature may be employed to get a white product similar to the commercial sample. The product is stable and highly acceptable.

Experiments were also conducted to determine the best processing method for peanut cake production. Ingredients for peanut cake were varied to obtain the best formulation. The final product was highly acceptable.

g. Bland Tasting Peanuts

The effect of soaking peanuts in different acid solutions in the production of bland tasting peanuts was determined. The method was found to be effective. The length of time of soaking has no significant effect on the characteristics of the product.

h. Peanut Milk Concentrate

The formulation and process for peanut milk concentrate was developed. The effect of pH of the soaking solution, different additives and flavoring, and dilution on the quality of the product was determined. Sensory evaluation showed that strawberry flavored concentrate obtained from peanuts soaked at pH 8.5 with added 0.2% carboxymethyl cellulose in the formulation was rated highest among treatments. The ratio of the concentrate to water was most acceptable at 1.3. Hot water grinding improved the acceptability of peanut milk concentrate. Soaking peanuts in sodium bicarbonate solution prior to grinding increased the soluble protein content of the product. However, it could also lower the product's acceptability.

i. Peanut cracker

Formulation studies were conducted to develop a peanut cracker. The acceptability of the product was not significantly affected by the type of flour used in the formulation. The optimum level of oil, starch and baking powder was determined. The product was highly acceptable.

j. Peanut Butter

The effect of varietal differences on the quality of peanut butter was determined. Peanuts were subjected to proximate analysis. Storage studies showed that the stability of peanut butter is greatly affected by the fat content of the peanut. Peanut was more prone to hydrolytic rancidity than to autoxidation.

k. Utilization of the Residue from Peanut Milk

Studies have been done to develop a peanut milk as a potential low cost, high-protein milk substitute. Peanut milk has been developed into fermented and fruit-flavored beverages, and used in the preparation of soft curd and vegetable-toned milk. In the process of extracting the peanut milk, a residue is left which is often discarded after filtering the milk. To maximize the utilization of peanuts, the residue can be developed into a nutritious food product which could be used as food supplement in countries where malnutrition is prevalent.

i. *Utilization of peanut residue as a base for cheese-flavored spread*

Peanut milk residue was utilized for the formulation of a cheese-flavored spread. Based on a_w and pH values, its stability was comparable with that of the commercial spread, thereby requiring refrigeration to prolong shelf-life. Processing into a spread provides a potential use for the peanut milk residue.

ii. *Development of pork and liver-flavored spreads from peanut milk residue*

Peanut milk residue was also used for the formulation of pork- and liver-flavored spreads. Chemical composition of the residue-based spreads is comparable to commercial samples. General acceptability scores ranged from 4.4 - 5.3 (7-point hedonic scale). Pork and liver flavors can be used even at 0.5% level of addition. From the results, it is possible to use peanut milk residue as a base for meat spreads.

iii. *Peanut milk residue as meat substitute*

The suitability of using peanut residue as substitute for meat in meat product preparations was also investigated in this study. Peanut meatloaves were prepared using boiled, defatted and peanut residue, subjected to proximate analyses, sensory evaluation and compared with commercial brands of meatloaf. General acceptability score for samples substituted with 50% peanut residue is 4.9 in the hedonic scale (1= unacceptable; 7 = acceptable). It is also comparable to the all-meat product (5.45) having a general acceptability score of 4.85. The product was processed in 211 x 300 cans at 121°C for 30 minutes. When compared with 2 commercial products, the peanut-substituted meatloaf is equally acceptable, with almost the same nutritional value and physical characteristics.

Peanut residue was also used to prepare 1 x 1" square nuggets. Proximate analysis on the sample showed it is a very good source of protein but it has high fat content. When subjected to sensory evaluation, peanut nuggets from residue was found to be very acceptable and has desirable flavor. This food product could be consumed as a snack, served with sauce.

3. Aflatoxin Studies in Peanut and Peanut Products

a. Aflatoxin Surveillance

The presence of aflatoxin in commercial peanut butter was monitored. Forty-eight samples of different brands of peanut butters and other peanut products were obtained from 12 outlets in the Philippines (Los Baños, Metro Manila, Cebu City and Cagayan de Oro City). Nine leading brands were monitored by collecting samples twice or thrice within the said period. Samples were analyzed for aflatoxin using both Pons method and Romer minicolumn method or Pons method alone. The water activity of samples were also determined.

Of the different peanut butter brands analyzed, six were positive in at least one sampling, while three brands (Lady's Choice, Gina and Kelly) were negative in all analyses. Among the contaminated peanut butters, one product was reported by BFAD to contain an alarming level of aflatoxin. Peanut products such as majareal, piñato, oven-baked peanuts and peanut brittle gave negative results. The water activity (a_w) of all the peanut samples was not correlated with the degree of their aflatoxin contamination.

b. Effect of Processing on Aflatoxin on Peanut

Sampling procedure and the most effective method of detoxifying aflatoxin were established. The effect of boiling, roasting (dry and oil roasting) and addition of baking soda and soda ash on the aflatoxin content of peanut samples initially contaminated with aflatoxin was investigated. Results indicated that among the three processes, oil roasting gave the highest average reduction, (88.3%) followed by dry roasting with 75.4% and boiling with 65.8%. Sodium carbonate was a more effective (66.7% - 69.7% reduction) soaking solution than sodium carbonate with a reduction of 36.51% - 48.85%.

The effect of processing as well as the dilution during processing on the detoxification of aflatoxin in contaminated peanuts was investigated. Based on calculated values, the effect of dilution is minimal compared with the destruction of aflatoxin due to processing.

c. Microbiological Control of Aflatoxin

A fungal isolate, *Cladosporium fulvum* was found inhibitory to the aflatoxigenic fungi *Aspergillus parasiticus*. Optimum media composition which can promote the growth of *C. fulvum* was determined. Toxicological and stability studies, development of a stable form of the metabolite produced by *C. fulvum* was done to determine the extent of its application. The metabolite was also isolated, purified and characterized.

C. fulvum grows favorably in the media containing malt extract as the carbon source and potassium nitrate as nitrogen source. Toxicological evaluation of water and oil soluble extracts from *C. fulvum* and its growth medium using chick embryo bioassay indicate minimal effect if any. Morphological assessment of the inhibitory effect of *C. fulvum* extract against *A. parasiticus* showed a decrease in spore production and constricted hyphae with increasing concentrations of the culture broth. Heat stability tests suggest that inhibitory activity is stable but colorimetric property is affected with change in hydrogen ion concentration.

The powdered and pelletized form of the metabolite produced by mixing culture broth with cassava flour, corn starch or rice flour as carriers was less potent than the culture broth per se due to the addition of the carrier. Another form developed by the addition of cornstarch with freeze dried culture broth is more potent that the culture broth. However, its efficacy in controlling growth of *A. parasiticus* in peanut is minimal due to its inability to totally coat the peanut surface.

The metabolite from *C. fulvum* can be extracted using charcoal in acidified culture broth and extraction with methanol or acetone. Purification done by HPLC yield a concentrated metabolite about 6.2 mg/L of the antibiotic. Initial spectroscopic studies indicate that the compound has a molecular weight of 274 and a tentative molecular weight of 274 and a tentative molecular formula of $C_{18}H_{26}O_2$.

C. Accomplishments at the Visayas State College of Agriculture (ViSCA)

1. Improved Interdisciplinarity

In its final year, the project sought the integration of a Development Communication Specialist for process documentation and to assist in catering to the needs of increased number of adopters. This was done in an effort to facilitate more rapid transfer of technologies and commercialization of peanut products.

2. Strengthening Enterprises and Markets: URIVIC and GWA

a. Improving Enterprise Operations

UNIVIC. The first step was to wean the group by transferring to a processing site in the PNRC settlement village closer to member's homes where they can operate independently, i.e., make decisions, schedules, process and market on their own. They were also trained on the job to improve record keeping, be conscious of processing hygiene, and grow in the spirit of cooperation. While the initial facilities investment are contributed by the PNRC, an easy payment plan was worked out. In return, the members render a paid service to PNRC and the latter also helps the processing group in product promotions. A product display area at the PNRC building was provided.

GWA. While the sweet potato-peanut cookies still have to find their niche in the market, other peanut products were introduced to the group in order to make their enterprise economically feasible. These included camote-peanut candy
(Camoreal) and "Kabkab" with peanuts.

b. Enterprise Development Training

Two conflict management sessions were conducted with GWA and URIVIC, and another with the other adopters. These were designed to improve the members' skills in coping up with group conflicts arising from personal and business circumstances using three different modules. This experience was highlighted with a field visit to four microenterprises in the south, three in Maasin and one in Bato. These visits stressed encounters by adopters with the way the existing indigenous processing enterprises were able to sustain and improve their operation through the years. In addition, the visit made possible the referral by the project of a "Bocarrillo" producer to a firm in Cebu to improve its product packaging. Bocarillo is a local delicacy made from cooked young coconut meat with milk.

c. Economic Benefits

Interaction between the cooperators and researchers enhanced the results of optimization processes. Optimization techniques and product quality resulted in greater economic advantages. Partial budget analysis showed that production of Lady Finger containing cassava flour using a pastry bag over the cookie molder had a net economic advantage of P1,619 per 20-kg of cassava flour bag mixture (Table 1). The new procedure with the use of pastry bag for molding showed a net advantage of P 754.09. This is due to the relatively higher product yield since cassava flour has a higher volume than wheat cake flour per given weight, a lower cost of cassava flour and a reduction in the cost of labor required for mixing and molding. Accounting for the effect of flour alone using the standardized cassava flour mixture, the advantage amounts to a P 147.58 over that of using pure wheat cake flour (Table 1).

Quality enrichment by adding peanuts to the Lady Finger formulation and producing button-shaped cookies increased the ROI index, using current prices, to 1.4 - 1.5 from the 1.3 - 1.4 of the Lady Finger at the cooperator

level. A positive net economic advantage of about P18 occurs for a triple recipe sample (Table 1). The added cost from peanuts and longer mixing time were more than offset by the increased yield and lesser molding time.

d. Improving Market Distribution Schemes

As the Lady Button and Lady Finger gained wider market acceptability in and outside Leyte, a distribution-collection scheme was established with the URIVIC group. This consisted of weekly production-distribution schedule for different areas and defined a minimum product-carry per area group at 600 packs per trip. The four area groups rotated each month included: Tacloban (5 outlets), Baby (3 outlets), Maasin and south of Leyte (2 outlets), and outside Leyte markets including Cebu, Manila and Los Baños. With five working days a week and production of 150 packs per day on the average, 750 packs can be produced weekly. Ormoc was served throughout with an extra 150 packs or more. One member of the group is in-charge of distribution outside Ormoc, while others are responsible for processing and distribution within Ormoc. Delivery was either on a cash basis or a term of one month, the latter corresponding to the distribution schedule per area.

In the plasticizer study, film was formed by deposition method at pH 9.0 and 90 /C. Four types of plasticizers at 3 levels of each were chosen to study, glycerol (G), sorbitol (S), propylene glycol (PG) and polyethylene glycol (PEG) at 1.96, 3.38 and 4.76% of film forming solution (or film forming solution: plasticizer were 100:2, 100:3.5 and 100:5, respectively). Film thickness, water vapor permeability (WVP), oxygen permeability (OP), tensile strength (TS) and percentage of elongation (% E) were measured. In sorption study, film was formed at pH 6.0, 7.5, 9.0 at 80°C (using 3% protein content solution). Glycerin 4.76% of film forming solution was used as plasticizer. Saturated salt solution were prepared to obtain 11, 33, 52, 75 and 93% RH, respectively. After drying for 16 hr film was cooled in desiccator for 15 min. Film was kept in desiccator for 7 days at 0% RH, 25°C and the thickness was measured; following which film samples were kept at 25/C and desired RH conditions. Thickness, a_w, and moisture content were determined after 9 days.

A transport scheme and packing process were also improved, with special consideration of the problem of breakage. Results revealed at 75% reduction of breakage when lady finger packages were placed in boxes with cardboard interlayer and almost no breakage if packs in jars.

Table 1. Partial budget analysis on process and product improvement activities.

a. Comparison between pastry bag and cookie molder using new procedure.
(For a 20-bag cassava flour mixture).

Added returns:		613.92	Added Costs:	0
Reduced costs:			Reduced return:	0
Labor:	Molding	425.73		
	Mixing	579.36		
Economic advantage		1619.01	Disadvantage:	0

b. Lady Finger using cassava flour, new procedure of mixing and pastry bag molding.
(For a 20-kg bag cassava flour mixture)

Added returns:		493.60	Added costs:	0
Reduced costs:			Reduced return:	0
Flour cost		P147.58		
Labor:	Mold	98.10		
	Mixing	14.81		
Economic advantage		1619.01	Disadvantage	0

c. Comparison of lady buttons and lady finger using tripled recipe of lady finger enriched with peanuts.

Added returns:		21.50	Reduced return:	0
Reduced costs:			Added costs:	
Labor:	Molding	1.24	Labor:Mixing	0.49
Economic advantage		22.74	Disadvantage	4.74

The main constraint, however, was the lack of supply of cassava flour due to technical problems faced by the flour processing cooperative. This hindered continuous processing and product deliveries, as well as preventing the intensive promotion and expansion of market outlets.

e. Raw Material Sourcing

In order to have a continuous supply of peanuts and link with product processors with peanut farmers, a map showing the key production areas in Region 8 was obtained from the Regional Office, Department of Agriculture, Tacloban City and given to the processors as guide.

In partnership with the GTZ project, the group was gradually trained to maintain their own records. The main constraint was the unavailability of the marketing and sustained processing activities.

f. Improving the Packaging Design

Five different label designs were computer-generated initially and printed in color combinations of ivory white, dull mustard, light yellow and brown. These were subjected to an informal design feedback test within 26 ViSCA respondents. During this initial test, the design with three-peanut-shaped windows was preferred slightly over a design depicting of the humanized peanut. Simplicity and daintiness of the former design was given is a reason for its preference. The latter was found, however, to be more attractive in color and symbolic. The three designs which were judged best were then subjected to further testing with the products using informal and structured methods.

Interviews by communication experts (n=10) with office workers (n=6) office workers at Los Baños n=10) and highly educated consumers (n=23) consisting of DAT-BAT teachers from Luzon, Visayas and Mindanao were

conducted. A focus group discussion was conducted in Ormoc with ordinary consumers (n=10) with relatively low level of education. The focus group consisted of consumers with ages for 13-46 years, 8 females and 2 males, 5 single and 5 married, 3 teenagers and 7 adults.

A label design pretest schedule used for ViSCA respondents was also used as a guide in the focused group discussion. Informal interviews were used with respondents outside ViSCA. These various methods were aimed to determine differences in responses elicited. Informal methods allowed for flexibility. For example, the FGD with the ordinary consumers allowed for greater interaction among the respondents and gave way to spontaneous suggestions.

For all groups, the humanized design was most acceptable among the three packaging designs due to its being attractive, informative, legible, convincing and a generally good and unique design, In particular, the respondent consumers of all educational levels said that the peanut cartoon appealed to all age groups and is more likely to attract buyers. The color combination was appropriate and the typefaces were very legible.

Some suggestions need to be considered with proper balance of taste, harmony and economy. The trained respondents suggested the use of simple yet appealing words or phrased such as snacks instead of delight, tasty and delicious instead of body-building nutrients and peanut-enriched instead of "peanut and peanut enriched". Also, additional information such as expiration date, nutritional facts and suggested storage (e.g., keep refrigerated) should appear on the packaging. A highlight color such as red may be evaluated, as well as green leaves, and the packaging material could be thicker. All groups suggested that each product should have its own associated unique design, e.g., the peanut cookies in bags with round windows, candies in bags with the humanized peanut, etc.

In the final analysis, respondents suggested that price is an important factor, though response tended to be influenced by the way a product is packaged. Good quality affordable local products can favorably compete with the brand products.

3. Product Enhancement and Diversification

a. Quality Enhancement of Lady Fingers and Lady Buttons

Although the R2 values were not that high, the effects of sugar and level of peanuts in products were highly significant ($P<0.05$) for all quality attributes. The shaded regions represent all combinations of peanuts and sugar in lady button formulations which produced products that met consumer acceptability scores of $6.5. Results suggest that the optimum region included a sugar level as low as about 35%, which could greatly reduce production cost without affecting the product acceptability. This information cannot be readily obtained if analysis done only ANOVA (data presented in Table 2). Response surface methodology (RSM) applied to factorial design was used in the process optimization of peanut beverage.

An enrichment study was conducted on lady button and the results are shown in Table 3. There were no significant differences among treatments in terms of percent moisture. However, differences were observed on the other components. The peanut supplemented lady buttons contained significantly the highest crude fat ($P<0.05$) which was about twice the amount in product containing soybean at the same level of enrichment. Soybeans contain about half the fat of peanut thereby explaining the results of the crude fat content of the product. The plain lady buttons had the lowest fat content. The products with either mungbean, soybean or peanut had significantly higher protein than the unsupplemented products. Legumes contain high protein content which raise the percent protein in the final products. Significant differences in terms of percent ash existed among the treatments.

b. Kabkab Process and Formulation Improvement and Optimization

Kabkab is a traditional puffed product made from cassava grates. Processing steps include peeling of cassava, grating, spreading, steaming and drying. Although these steps are simple, they are labor intensive considering that the raw material is grated cassava. Spreading and cooking are the most labor intensive steps. Studies showed that spreading grates in between cellophane sheets with a rolling pin was about 400% more efficient than hand spreading. Steaming instead of blanching in boiling water improved the cooking efficiency by 300%, which consequently reduced the drying cost by about 50%. Using the fabricated steamer instead of an ordinary steamer also reduced cooking cost by about 50%. The nutritional value of kabkab is enhanced by adding peanut, mungbean or soybean. To optimize its formulation, mixture response methodology was used.

Table 2. Mean taste panel scores of Lady buttons with different amounts of sugar and peanut.1/

Treatments		Sensory Qualities				
Sugar	Crushed					Overall
30	0	7.0abc	6.7dc	5.8d	5.9c	6.1d
40	0	7.5a	7.4ab	7.2ab	7.4ab	7.3ab
50	0	7.1ab	7.5a	7.7a	7.7a	7.7a
30	10	7.1ab	6.8bcd	5.6d	5.9c	6.2cd
40	10	7.0abc	7.3ab	6.8bc	6.9b	6.9b
50	10	6.6bc	7.0abc	7.0abc	7.3ab	7.1ab
30	20	7.1ab	6.4d	5.6d	5.8c	5.8d
40	20	6.8bc	7.0abc	6.6c	6.8b	6.8bc
50	20	6.5c	6.8cd	7.3b	7.2ab	7.2ab

1/ Means in columns followed by the same letters are not significantly different using DMRT at 5% level of significance.

Table 3. Proximate composition of Lady Buttons with different levels and combination of peanut, mungbean and soybean.

Treatments			% Moisture (NS)	% Crude Fat	% Crude Protein	% Ash
Mungbean	Soybean	Peanut				
0	0	0	4.0	5.4c	8.4f	1.4e
0	10	0	3.8	6.5bc	10.3cd	1.5de
0	20	0	3.7	6.5bc	11.6b	1.7ab
10	0	0	3.8	5.4c	9.4e	1.4e
10	10	0	3.3	6.3bc	10.7c	1.6bdc
10	20	0	3.5	7.6b	11.9b	1.7ab
20	0	0	3.2	5.6c	9.9de	1.6bcd
20	10	0	3.2	6.4bc	11.7b	1.6bcd
20	20	0	3.8	7.8b	13.2a	1.9a
0	0	0	3.2	11.0a	10.4cd	1.4e

1/ Means in columns followed by a common letter are not significantly different at 5% level of significance using DMRT.

NS = Not significant.

c. Packaging and Shelf Life Studies of Peanut-root Crop Baked Products

Lady Finger, lady buttons and peanut-sweet potato (PS) cookies were stored under ambient (30°C, 75% RH) conditions. The three products were packaged in 17.5 micron (0.00069 inch) thick polypropylene and stored in an incubator at 30°C. Packaged products stored for various periods were subjected to sensory evaluations. Sensory evaluation of the products were conducted to assess the loss in quality of samples, with respect to loss in texture. A sensory score for texture of 7 was considered the cut-off point for acceptability as pointed

out in critical moisture content determination. The results gave shelf lives of 12 days for lady finger, 17 days for lady buttons and 20 days for PS cookies. The predicted accelerated shelf life for lady finger, lady buttons and PS cookies were 1.6, 2.2 and 2.0 days, respectively. Hence multiplication factors for converting the shelf life of the products at accelerated conditions were 7.5 for lady finger, 7.7 for lady buttons and 10.2 of PS cookies. This compares well with the multiplication factor of 7-10 for commercial dry snack products.

By using a packaging equation, the packaging requirements of lady finger, lady buttons and PS cookies can be determined. The calculated package thickness is based on accelerated conditions of 38°C and 90% RH. Hence to translate this into ambient conditions (30°C and 75% RH), a multiplication factor was used. A shelf life of one month at ambient conditions would be 3 to 4 days at accelerated storage conditions. Using the same packaging material, bag dimensions and sample characteristics, the thickness of the bag was obtained (Table 4). Results show that lady finger needs to be packaged in a 91.4 microns (0.0036 inch) thick polypropylene bag to achieve the same shelf life of about one month at ambient temperature. Lady buttons need to be packaged only in a 50.8 microns (0.0020 inch) thick of polypropylene bag to achieve the same a shelf life at ambient temperature; for PS cookies, the required thickness of the polypropylene bag is only 22.9 microns (0.0009 inch) thick. Based on commercially available thicknesses of polypropylene bags available in Metro Cebu (Table 5), the thickness of the bags for lady finger, lady buttons, and PS cookies should be 95.0 microns (0.00374 inch), 54.9 microns (0.00216 inch) and 24.9 microns (0.00098 inch), respectively.

4. Increased Number of Adopters

The project expanded the number of adopters by introducing new groups with peanut-enriched product: the Bubon Integrated Root Crop Processing Cooperative with "chocass"; the San Agustin Rural Improvement Club with SP-peanut candy; ALEA with peanut-flavored Polvoron; and a private entrepreneur with peanut/mungbean-enriched "kabkab".

a. BIRPA (Bubon Integrated Root Crop Producers Association)

A root crop processing group of 12 married couples in Bubon, Baybay, Leyte has taken on the name 'Bubon Integrated Root Crop Producers Association.' Most have been educated up to the elementary level, and are 40 years of age or older. The group organized themselves in 1984 with support and assistance from ViSCA and started operations in 1988. They are known for their delicious cassava chocoroll (chocass) in Leyte. BIRPA was also a recipient of the two schools-on-air on swine and coconut production conducted by VICARP-Regional Communication and Training Unit (VICARP-RCTU) based at ViSCA in cooperation with ViSCA Radio DYAC.

With their zeal and enthusiasm, BIRPA members wanted to engage in processing other root crop products. This was when the UGA-ViSCA immediately responded to their request for training on how to make kabkab from cassava grates enriched with peanuts or mungbean. In 1990, they were also taught on how to make a takudo waffle. Because they already had gained skills from previous training, four couples separated from the group and established their own livelihood in 1992. The remaining eight couples have strengthened and they continued producing and marketing the peanut-enriched chocass, chippy, and kabkab.

Fixed assets of BIRPA are currently P9,000 of which is initial capital. The UGA-ViSCA peanut CRSP project has been collaborating with DOST to procure some equipment and construct a concrete processing center in Bubon. Their association is now a member of Leyte Countryside Entrepreneurs and Exporters Association organized by the Department of Trade and Industry. The provincial government is building a Resource or Trade center which will have a permanent show window and market center, and BIRPA will be the distributor through one of is members.

Table 4. Packaging material thickness needed for a shelf life of 30 days for lady finger, lady buttons and PS cookies at ambient conditions (Assumptions: M_i=1.20% d.b.; Weight of product = 35 g; Package dimensions: 0.15m x 0.125m).

Packaging Material	Thickness, microns (inch)		
	lady finger	lady buttons	PS cookies
Low Density Polyethylene	120.0 (0.0047)	67.6 (0.0027)	29.7 (0.0012)
High Density Polyethylene	39.3 (0.0015)	22.2 (0.0009)	9.7 (0.0004)
Polypropylene (Ordinary)	92.1 (0.0036)	51.9 (0.0020)	22.8 (0.0009)

Table 5. Packaging materials that are commercially available in Metro Cebu and their corresponding thicknesses.

Packaging Material	Thickness	
	(microns)	(inch)
Polypropylene (Ordinary)	17.5	0.00069
	24.9	0.00098
	30.0	0.00118
	50.0	0.00197
	54.9	0.00216
	95.0	0.00374
Low density polyethylene	62.5	0.00246
High density polyethylene	76.2	0.00030
	24.9	0.00098

The vision of BIRPA is to nurture and pass on technology to their children and grandchildren because they can not simply let go of a livelihood which gave them a sense of achievement. The activity also gives them additional income to meet their day to day needs, help send their children to school, and pay for house appliances and kitchen wares.

b. SARIC (San Agustin Rural Improvement Club)

The San Agustin Rural Improvement Club (SARIC) is a Department of Agriculture (DA)-assisted women's group in San Agustin, Baybay, Leyte, a few kilometers away from ViSCA, which had stopped their activities when the DA technician was reassigned to another location. With the initiative of the group's leader, they requested The UGA-ViSCA project for training on the processing of sweetpotato-peanut candy. Subsequent training helped them revive in 1995, and they are now actively involved in processing. Through the project, they were also trained in processing other food products such as binangkal and doughnut using a composite of cassava flour to make their enterprise economically viable.

SARIC also produces crocheted abaca fibers which it supplies URIVIC and ViSCA bakeshop to improve packaging of lady button and lady fingers in jars. One of their constraints is the lack of a processing facility,

and equipment. At the moment, SARIC are using the food processing facility at ViSCA which serves as the incubation facility.

c. Grace Mae Food Products

To expand coverage of the project to the southern part of Leyte Island and to get more impact, information was gathered from the DA in Tacloban City concerning peanut processing areas of the region. Work has initiated with the Balistoy family in Maasin, who owned a peanut butter and peanut product enterprise. They started their enterprise in 1987 with 6-ganta of peanut for peanut butter. Now they process 20 sacks of peanuts each week and have hired two additional workers and sourced out imported peanuts from Cebu City.

Initially, the Department of Social Welfare and Development (DSWD) provided them capital (P5,000). They have expanded their market in Leyte and in Mindanao. Mrs. Victoria Balistoy has indicated her interest to also engage in the production of peanut/mungbean-enriched kabkab with her neighbors. The project has responded to her request and is extending technology to a women's group organized by her.

d. ALEA

Peanut-enriched polvoron and cassava cookies are processed by a group of four women in the Municipality of Inopacan, Leyte. The group's name came from their first names (Aida, Lita, Emma, and Amparo). Through the project, these women learned skills in processing these products. They were able to loan out P5,000 for their capital as well as reloan for about P16,000 from DSWD for purchasing an oven and other processing tools. Negotiations are in progress with the provincial government to build a processing center which ALEA can use together with the other food processors in the municipality.

5. Expanded Collaboration

Collaboration with the Department of Science and Technology (Region 8) based in Tacloban City operations of the BIRPA. A project proposal was submitted to the DOST for BIRPA to avail of a loan-grant for capital equipment investment of P50,000.

Linking with the local DSWD office in Inopacan, Leyte made assistance to the ALEA processing group possible. This office helps monitor the group's activities, while ViSCA is charged with the technicalities of processing and market as well as enterprise strengthening.

6. Product Promotions

The project produced two posters for the Federation of Crop Science Society of the Philippines annual scientific conference held on May 13-18, 1996 in Davao City. Likewise, the project will be promoting its technology through multi-media opportunities. This will be in the form of slides, radio plugs, posters, and a video.

7. Process Documentation

With the release of funding in late December 1995, the project was able to hire an affiliate staff from the Department of Development Communication at ViSCA to work on the process documentation of the project. The team is composed of a development communication specialist, a socio-economist, a food engineer, and a food technologist visited the cooperators and documented their activities. Some follow-ups were also done to answer cooperators' problems concerning adoption of the technologies. The project staff always views this activity as a transfer of the technology. An increase in the number of adopters encouraged project staff to continue its task of helping small processors augment their incomes.

8. Project Documentation and Product Promotional Materials/Activities

A guide was made available to cooperators using basic recipes. Project documentation activities included the preparation of slides on the activities of the cooperators in product marketing and distribution channels. Permanent documentation in the form of an album was prepared to record activities of the project, including

training and cooperators' activities. Significant activities of the project and cooperators were documented in a video and will serve as promotional material for peanuts and peanut enriched-products.

D. Accomplishments at the University of Georgia (UGA)

1. Peanut Butter and Honey Product

a. Development and Formulation Optimization

The objectives of the study were to formulate prototypes of honey-peanut butter (HPNTB), to optimize the formulation of HPNTB for consumer acceptability and to characterize flavor, texture and appearance of the optimized HPNTB formulation.

Shelled, U.S. No. 1 "medium runner-type peanuts" (1993 crop) were used in the preparation of peanut butter. A 3x3 factorial design was used to investigate the effect of honey and salt level on sensory properties of peanut butter. Formulations containing three different levels of dry honey powder (Sweet'n'Neat^R 2000 ADM Arkady, Olathe, KS) and salt were used. Dry honey was used to minimize flavor fade and other problems with stability resulting from increase water activity. The lowest level (6.0%) was selected because it resulted in a product that conforms to the standard of identity of peanut butter. The higher levels represent honey peanut butter spreads which do not conform to the standard of identity of peanut butter but compare in some ways, to peanut butter and jelly products which exist in the market. In addition to the dry honey (6, 13 and 20%) by weight of ground roasted peanuts and salt (1, 1.5 and 2%), a commercial stabilizer (Fix-X, Procter & Gamble, Cincinnati, OH) was used at 1.25% by weight of ground roasted peanuts. The control peanut butter was prepared using 6% corn syrup solids (Georgia Spice Company, Atlanta, GA), 1.25% Fix-X and 1% salt by weight of ground roasted peanuts.

Consumers were recruited by telephone from a consumer database maintained at the sensory laboratory and from a list of faculty, students and staff at the University of Georgia, Agricultural Experiment Station, Griffin, GA to evaluate peanut butter containing honey. Participating consumers (n=93) were, screened to be regular consumers of peanut butter (at least eat peanut butter twice of a week), between 18 and 65 years of age, and available and willing to participate. Consumers were selected to represent a variety of educational backgrounds, marital status, household incomes, and employment status. The test was conducted in a sensory laboratory under white incandescent lights in partitioned booths. Consumers were asked to determine acceptability of color, spreadability, sweetness, saltiness, flavor, texture, and overall acceptance of peanut butter containing honey and a control (regular peanut butter) using a 9-point hedonic scale (1 = 'dislike extremely', 5 = 'neither like nor dislike', and 9 = 'like extremely').

Three to five consumer test sessions were conducted over a period of three consecutive days. For each session, each consumer evaluated five samples then had a compulsory 10 min break and evaluated another five samples. The samples were presented to consumers in 1-oz plastic cups coded with 3-digit random numbers and served monadically with half a slice of white bread with crusts removed. Consumers were instructed to spread peanut butter on the bread to evaluate spreadability and sample it to evaluate the other attributes.

The data was analyzed using the Statistical Analysis System software package. Prediction models for all attributes except color were developed using regression analysis (PROC REG). The models were used to generate contour plots for spreadability, sweetness, saltiness, flavor, texture and overall acceptability. The optimization boundary for HPNTB was set at a score of 6.0 (= 'like slightly') or greater.

Ninety-three consumers participated in this study. Seventy-seven percent of the participants were females because the database used for recruiting represents the person in a household who purchase and prepare food in the household. The panel was composed of Caucasians (72%). Married panelists made up 62% of the sample. Most participants had completed high school and 58% had either attended college courses, completed college, or obtained a higher degree. About 63% of the participants were employed full time, and the median annual household income of the panel was between $30,000 to $39,000.

Mean values for consumer scores for the control and HPNTB containing varying proportions of dry honey and salt were compared in Table 6. The control had a texture rating (x = 6.8) significantly higher ($p<0.05$) than all HPNTB treatments. The treatment containing 20% dry honey and 2% salt treatment had the lowest color rating (x=6.2) which was significantly different ($p<0.05$) from the control. HPNTB containing 6% dry honey and 1% salt was not significantly different from the control except for texture (x=6.1). HPNTB containing 13% dry honey and 1% salt were significantly different from the control ($p<0.05$) for spreadability, texture, and overall acceptability. Spreadability, texture, flavor, and overall acceptability scores of HPNTB containing 20% dry honey and 1% salt were significantly different from the control ($p<0.05$).

Table 6. Mean consumer scores for acceptability of honey peanut butter and a control (n = 93)

% by weight of ground peanut		**Acceptability Score**						
Honey	**Salt**	**overall**	**color**	**spreadability**	**sweetness**	**saltiness**	**flavor**	**texture**
6	1.0	6.0ab [y]	6.7a	7.0ab	6.0a	6.0ab	6.0ab	6.1b
6	1.5	5.6bc	6.6ab	6.8bc	5.5bc	5.5bc	5.5bc	5.7bc
6	2.0	5.2cd	6.6a	6.5bcd	5.2c	4.9d	5.1c	5.6bc
13	1.0	5.8b	6.7a	6.4cd	6.0ab	6.0ab	6.0ab	5.3cde
13	1.5	5.6bc	6.6ab	6.6bc	5.8b	5.6b	5.8b	5.7bc
13	2.0	5.0d	6.6ab	6.1de	5.0c	4.7d	5.1c	4.8ef
20	1.0	5.6bc	6.5ab	6.0de	5.9ab	5.8ab	5.7b	5.0def
20	1.5	5.7bc	6.6a	5.9ef	5.9ab	5.8b	5.9b	5.4cd
20	2.0	4.8d	6.2b	5.6f	5.1c	5.0cd	5.0c	4.6f
control [y]		6.4a	6.8a	7.3a	6.2a	6.2a	6.5a	6.8a

\x A 9-point hedonic scale was used (1 = 'dislike extremely', 5 = 'neither like nor dislike', and 9 = 'like extremely').
Mean values in the same column that are not followed by the same letter are significantly different (% = 0.05).
[y] control = peanut butter without honey, containing 6% corn syrup solids and 1% salt.

Regression analysis results and the F-statistic were used to determine which reduced model was not significantly different from its corresponding full second order model. The best fitting models were used to generate contour plots. The contour plots indicated that increasing salt percentage decreased the sweetness, flavor and saltiness ratings of the HPNTB. Spreadability, texture and overall acceptability rating decreased with increasing dry honey and salt percentage of HPNTB. The model for overall acceptance as a function of dry honey and salt (Overall acceptability = $7.0 - 0.02\ x_1 - 0.85\ x_2$, where x_1 = dry honey level and x_2 = salt level) showed that HPNTB containing 7% dry honey and 1% salt will have the rating of 6 (= 'like slightly').

Product formulations that scored 6.0 (= 'like slightly') or higher were used to determine the combination of ingredient levels that would result in optimum formulations. The combination of formulations which could result in scores of 6.0 or higher were shaded then superimposed. The areas of overlap (score = 6.0 or higher) were outlined.

From prediction equations, a HPNTB containing 7% dry honey and 1% salt would have rating score of 6.0 or higher for all the attributes. HPNTB containing 7% dry honey and 1% salt would not be significantly different

from the control for all the attributes except texture. Texture differences were found because the dry honey produced grainy mouthfeel.

b. Shelf Stability Studies

Objectives were to determine the shelf stability of the HPNTB compared to a control peanut butter (PNTB) containing no honey. The honey peanut butter formulation optimized above was used in this study. HPNTB containing 7% dry honey powder, 1% salt, and 1.25% stabilizer and PNTB were prepared in duplicate. HPNTB and PNTB were stored at 25°C and 50°C. Samples were removed from storage and evaluated every week (for 12 weeks) then every month (after the 4th, 5th and 6th months of storage) by sensory descriptive analysis.

Fourteen panelists were recruited from a list of trained panelists who had previously participated in descriptive analysis tests at the sensory laboratory, of the Center of Food Safety and Quality Enhancement, the University of Georgia. Criteria for recruitment of panelists were that they were: (1) between 18 and 55 years of age, (2) non-smokers, (3) not allergic to peanut butter, (4) consumers of peanut butter at least twice per week, (5) willing to evaluate peanut butter and (6) available and willing to participate during training and test dates.

Panelists were given additional training for at least eight hours. During the training, panelists were presented with several peanut butter samples, such as control peanut butter and HPNTB. Descriptive terms that characterize the sensory properties of the samples were developed by the panel. The final list of attributes and their definitions to be used during calibration and evaluation were decided upon by panel consensus (Table 7).

The panelists were trained to rate the intensity of stimuli by presenting them with standard references for the basic tastes. Panelists were presented with peanut butter samples and selected reference standards to evaluate as a group to assure that judgement of each panelist was calibrated against the other panelists. A control peanut butter used as a warm-up sample was rated at each training session. Intensities of the warm-up sample attributes, were agreed upon by panel consensus.

Panelists evaluated the samples under white incandescent lights in partitioned booths equipped with computer terminals in a sensory laboratory. Samples were coded with three-digit random numbers and presented to the panelists monadically. Panelists were instructed to calibrate their judgement using standard references, then evaluate the warm-up sample and compare responses to intensity ratings agreed upon by the panel during the training (posted on each booth) and finally to evaluate the samples. Panelists were instructed to spread peanut butter on half a slice of white bread to evaluate spreadability and adhesiveness. Color was evaluated separately under a light box which simulated day-light condition (Model EBA-220, Examolite Daylight with 2-20 W, F20T12 EXX Deluxe Examolite fluorescent tubes, MacBeth Corporation, New York, NY). Sample order of presentation was randomized for each panelist at every session. Water and unsalted crackers were provided for panelists to cleanse their palates between samples.

Unstructured line scales were used to rate samples for intensities of each attribute. Panelists recorded their responses directly onto a computer utilizing an interactive program designed to prompt them to rate one attribute per sample at a time. The line scales consisted of 150-mm lines with a marker that was moved from end to end with a cursor key. Panelists marked each scale to indicate their rating of intensities of each attribute. Each sample was evaluated twice by each panelists.

The data was analyzed using the Statistical Analysis System (SAS, 1985) software package. Cluster analysis (VARCLUS) was conducted to determine panelist performance and to detect outliers. Analysis of variance (ANOVA) was used to detect significant differences ($p<0.05$) due to the independent variables (honey and salt). The Least Significant Difference (LSD) test was performed.

Characteristics of freshly processed HPNTB and PNTB are presented in Table 8. Appearance characteristics of HPNTB were identical to that of regular PNTB which contained no honey. Both peanut butters had

intensities for color, creamy and oily which were not significantly different. HPNTB and PNTB had almost the same intensities for roasted peanutty, raw beany, dark roast, oxidized flavors. HPNTB had slightly higher sweet and bitter intensities and lower salty intensity compared to PNTB, but the differences were not significant ($p<0.05$). Texture characteristics of HPNTB were similar to the PNTB. Although HPNTB had slightly lower intensities for oiliness, smoothness and spreadability and slightly higher intensity for adhesiveness, the differences were not significant ($p<0.05$).

Color, creaminess and oily intensities of HPNTB stored up to six months at 5°C did not change. At 25°C HPNTB had no change in color, creaminess and oily appearance. Intensities for appearance attributes of HPNTB were not different from HPNTB at 5°C or PNTB at 25°C. At 50°C HPNTB had a darker color than when stored at 25 or 5°C because high temperature accelerated the Maillard reaction between amino acids and reducing sugars in honey. HPNTB was significantly ($p<0.05$) darker after 28-174 days of storage. After 21 days of storage HPNTB had darker ($p<0.05$) color than HPNTB at 5 or 25°C and PNTB at 50°C. At 50°C HPNTB had no significant change in the creaminess and oily appearance. HPNTB was not different from HPNTB at 5 or 25°C and was slightly less creamy than PNTB at 50°C. HPNTB was not different from 5 or 25°C but was slightly less oily than PNTB at 50°C.

Raw beany, oxidized, and cardboard flavor intensities of HPNTB and PNTB stored at different temperatures for six months are presented in Table 9.

Table 7. Definition of terms used in evaluating honey peanut butter

Term	Definition
Appearance	
Color	The actual color name or hue
Creamy	Amount of gritty particles present in peanut butter.
Oily	The appearance of glossy sheen on peanut butter.
Aromatics	
Raw beany	Aromatic associated with raw peanut.
Roasted peanutty	Aromatic associated with medium-roasted peanut.
Dark roasted peanut	Aromatic associated with dark roasted peanut and having very browned or toasted character.
Cardboard	Aromatic associated with wet cardboard.
Oxidized	Aromatic associated with stale peanut.
Tastes	
Sweet	Taste associated with sugar.
Bitter	Taste associated with caffeine.
Salty	Taste associated with salt.
Textures	
Oiliness	Degree to which free oil is perceived in the mouth.
Smoothness	Degree to which grains or granules are not perceived in the mouth.
Adhesiveness	Force required to remove peanut butter from the palate during normal eating process.
Spreadability	The ease with which peanut butter spreads.

Table 8. Characteristics of honey peanut butter (HPNTB) and peanut butter (PNTB) as evaluated by a trained panel[x]

	Appearance[y]			**Aromatics**[y]					**Tastes**[y]			**Texture**[y]s			
Sample	CLR	CRMY	OLY	RABNY	RSTP	DRKR	OXDZ	CRDBD	SWT	SLTY	BITR	OLNS	SMTH	ADHS	SPRD
PNTB	64.2[z]	66.4	88.4	4.8	60.6	33.9	4.7	0.8	36.7	39.0	1.5	42.6	71.5	59.7	74.6
HPNTB	64.4	67.3	87.5	6.1	61.4	34.5	4.9	1.5	43.8	37.2	3.3	39.7	68.5	61.9	73.6

[x] Values reported are means of 26 observations (2 replications from 13 panelists) using 150 unstructured line scale.

[y] CLR = color, CRMY = creamy, OLY = oily, RABNY = raw beany, RSTP = roasted peanutty, DRKR = dark roast, CRDBD = cardboard, SWT = sweet, SLTY = salty, BITR = bitter, OLNS = oiliness, SMTH = smoothness, ADHS = adhesiveness, and SPRD = spreadability.

[z] Mean values in the same column followed by different letters are significantly different (p#0.05).

Table 9. Mean ratings for raw beany, oxidized, and cardboard flavor intensities of honey peanut butter (HPNTB) and peanut butter (PNTB) during storage at different time as evaluated by a trained panel[x]

Storage Time (days)	PNTB (25°C)			HPNTB (25°C)			PNTB (50°C)			HPNTB (50°C)			HPNTB (5°C)		
	RBN[y]	OXD	CRB	RBN	OXD	CRB	RBN	OXD	CRB	RBN	OXD	CRB	RBN	OXD	CRB
0	4.8	4.7	0.8	6.1	4.9	1.5	4.8	4.3	0.0	6.1	4.7	1.5	6.1	4.9	1.5
7	5.2	0.8	0.0	3.0	2.5	0.2	5.2	5.2	0.4	5.5	7.2	0.7	3.4	0.8	0.0
14	0.3	0.3	0.4	2.2	0.4	0.1	0.3	2.6	0.5	2.9	0.9	1.9	0.3	0.2	0 . 1
21	4.2	0.1	0.0	4.2	0.2	0.0	4.4	0.2	0.0	3.8	1.7	0.7	3.7	0.2	0 . 0
28	0.5	0.5	0.0	0.1	0.1	0.0	2.3	1.9	0.0	0.4	1.0	0.8	0.1	0.0	0.0
35	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.4	0.0	3.1	0.0	0.0	0.2	0.0	0.0
42	0.0	0.0	0.0	0.4	0.0	0.0	2.0	0.3	1.3	1.0	0.1	2.5	0.0	0.0	0.1
49	0.4	0.0	0.8	0.0	0.1	0.0	1.7	1.1	3.8	0.5	1.0	2.3	0.1	0.2	0.1
56	1.2	0.0	0.0	0.1	0.0	3.2	0.6	0.2	4.1	0.8	0.1	3.7	0.5	0.0	0.1
63	0.1	0.0	0.0	0.2	0.2	0.0	0.4	2.2	3.6	0.5	3.8	3.2	0.1	0.5	0.1
70	0.8	0.0	0.0	0.3	1.2	3.7	0.7	2.3	2.3	0.6	3.5	3.6	0.0	0.0	0.0
77	1.2	0.0	0.0	0.1	0.1	0.0	0.6	0.9	1.0	1.0	3.0	2.0	0.0	0.2	0.0
84	0.4	0.6	0.3	2.0	0.0	0.0	0.4	1.8	0.9	0.8	3.1	2.8	0.0	0.2	0.2
114	3.4	3.1	1.0	1.1	3.9	0.3	6.9	10.5	0.5	7.5	8.0	4.7	0.6	1.1	0.0
144	1.3	0.3	0.0	2.4	0.3	0.0	2.8	5.4	1.1	0.0	3.8	1.6	0.2	0.6	0.0
174	5.3	0.0	0.0	4.7	0.0	0.1	1.9	6.7	1.1	6.1	3.0	1.3	4.4	0.2	0.1

[x] Values reported are means of 24 observations (2 replications from 12 panelists) using 150-mm unstructured line scale.

[y] RBN = raw beany, OXD = oxidized, CRB = cardboard.

Table 10. Mean ratings for sweetness, saltiness and bitterness of honey peanut butter (HPNTB) and peanut butter (PNTB) during storage at different time as evaluated by a trained panel[x]

Storage Time (days)	PNTB (25°C)			HPNTB (25°C)			PNTB (50°C)			HPNTB (50°C)			HPNTB (5°C)		
	SWT[y]	SLT	BTR	SWT	SLT	BTR	SWT	SLT	BTR	SWT	SLT	BTR	SWT	SLT	BTR
0	36.7	39.0	1.5	34.8	37.2	3.3	36.8	39.0	1.5	34.8	37.2	3.3	34.8	37.2	3.3
7	33.1	37.8	1.2	31.4	37.5	2.5	32.4	34.8	1.2	31.3	36.8	4.2	36.0	38.9	0.6
14	32.7	39.9	0.4	33.4	39.0	1.8	34.9	38.9	0.7	34.3	38.2	0.3	33.6	39.9	0.1
21	32.9	40.1	0.1	34.6	38.5	0.0	33.1	38.9	0.2	33.2	39.0	0.9	34.6	39.4	0.0
28	33.8	38.8	0.1	33.3	38.1	0.1	35.3	38.6	0.3	34.9	39.0	0.2	35.7	40.0	0.1
35	35.5	38.3	0.0	33.8	40.1	0.0	34.4	39.2	0.1	34.1	39.7	0.3	35.4	39.6	0.0
42	34.4	38.5	0.0	34.1	38.8	0.2	33.8	39.1	0.3	34.8	38.7	0.2	35.0	40.3	0.0
49	33.2	38.1	0.1	34.1	39.7	0.2	33.4	37.9	0.2	34.7	37.9	0.5	34.8	38.3	0.0
56	33.9	39.2	0.0	34.0	38.7	0.0	33.9	38.4	0.5	34.0	38.0	0.5	35.0	38.1	0.3
63	34.2	39.5	0.2	34.2	39.4	0.0	33.2	38.5	0.3	33.1	39.0	0.6	35.1	40.5	0.0
70	34.3	39.0	0.2	33.6	38.2	0.1	34.2	38.2	0.7	33.3	38.2	1.1	35.3	38.6	0.0
77	34.1	38.7	0.0	33.7	38.8	0.0	32.8	38.9	0.4	32.3	38.1	0.6	34.3	39.7	0.0
84	34.2	38.8	0.3	34.9	39.3	0.3	33.6	39.2	0.8	34.1	38.6	1.5	34.9	39.2	0.0
114	32.9	37.7	0.8	32.5	39.1	1.0	31.5	38.1	2.2	31.6	37.6	6.7	34.9	40.5	0.1
144	33.8	39.7	0.3	34.4	38.5	0.3	34.2	39.6	0.7	31.2	38.9	2.9	34.9	39.9	0.0

[x] Values reported are means of 24 observations (2 replications from 12 panelists) using 150-mm unstructured line scale.

[y] SWT = sweet, SLT = salty, BTR = bitter.

HPNTB and PNTB samples had similar roasted flavor intensity ranging from 55 to 61. All samples had very low intensities for raw beany flavor ranging from 0 to 8, cardboard flavor ranging from 0 to 5. Oxidized flavor did not develop in any of the samples. The ranging of intensity in all samples was from 0 to 10.

At 5°C HPNTB stored for six months, had no change in the raw beany, roasted peanutty, dark roasted, oxidized and cardboard flavor intensities. Storing HPNTB at 25°C for six months had no effect on the roasted peanutty flavor. Raw beany, dark roasted, oxidized and cardboard flavor intensities were not increased when HPNTB was stored at 25°C for six months. HPNTB was not different from HPNTB at 5°C or PNTB at 25°C for roasted peanutty, raw beany, dark roast, oxidized and cardboard flavor intensities.

Roasted peanutty intensity of HPNTB stored at 50°C for 0-56 days decreased ($p<0.05$) and remained unchanged from 56-114 days then increased. Roasted peanutty intensity of HPNTB after 56 days of storage was less ($p<0.05$) then HPNTB at 5 or 25°C, but was not different from PNTB at 50°C. At 50°C raw beany flavor intensity of HPNTB remained low (= 8 out of 150) and did not change when stored for six months. Raw beany flavor intensity of HPNTB stored for six months at 50°C was not different ($p<0.05$) from HPNTB stored at 5 or 25°C and PNTB at 50°C. Dark roasted flavor intensity of HPNTB was higher ($p<0.05$) after 42 days of storage at 50°C. Dark roasted flavor of HPNTB stored for six months was higher ($p<0.05$) than HPNTB stored at 5 or 25°C and PNTB at 50°C.

Oxidized flavor scores of HPNTB increased significantly only after 114 days. Oxidized flavor intensity of HPNTB stored for 114 days was higher ($p<0.05$) then HPNTB stored at 5 or 25°C and lower than PNTB at 50°C, but was not significantly different from PNTB at 50°C (Table 9). Cardboard flavor intensity of HPNTB stored for six months at 50°C was not significantly different ($p<0.05$) than HPNTB at 5 or 25°C and PNTB at 50°C.

Sweetness, saltiness, and bitterness intensities of HPNTB and PNTB stored at different temperatures for six months are presented in Table 10. At 5°C HPNTB had a change in the sweet, salty and bitter tastes. HPNTB stored at 25°C had no change in the sweet, salty and bitter tastes. The sweet, salty and bitter intensities of HPNTB was not different from HPNTB at 5°C or PNTB at 25. At 50°C HPNTB had no change in the sweet and salty tastes. HPNTB stored at 114 days was less sweet than HPNTB at 5°C, but not different from HPNTB at 25°C or PNTB at 50°C. HPNTB stored 114 days was less ($p<0.05$) salty than HPNTB stored at 5°C but not different from HPNTB at 25°C or PNTB at 50°C. The bitter taste of HPNTB increased ($p<0.05$) after storage for 114 days then decreased. Bitter intensity of HPNTB stored 114 days was high ($p<0.05$) than HPNTB at 25 or 5°C and PNTB at 50°C.

Oiliness, smoothness, adhesiveness, and spreadability intensities of HPNTB and PNTB stored at different temperatures for six months were determined. At 5°C HPNTB had no change in the oiliness, adhesiveness and spreadability from 0 to six months of storage. The smoothness intensity however increased significantly ($p<0.05$) after 14 days of storage then remained unchanged for up to six months. At 25°C HPNTB had no change in oiliness, adhesiveness and spreadability. The smoothness intensity of HPNTB increased significantly after 14 days of storage then remained unchanged for up to six months. HPNTB was not different from HPNTB at 5°C but was less oily compared to PNTB at 25°C. HPNTB was less smooth than PNTB at 25°C and HPNTB at 5°C. HPNTB was not different from HPNTB at 5°C but was more adhesive than PNTB at 25°C. HPNTB was not different from HPNTB at 5°C but was less spreadable than PNTB at 25°C. At 50°C oiliness intensity of HPNTB decreased significantly ($p<0.05$) after 42 days of storage and remained unchanged up to 84 days then increased significantly ($p<0.05$) after 114 days then decreased. HPNTB had lower oiliness intensity compared to PNTB stored at 50°C. After 42 days of storage HPNTB had less oily texture than when stored at 25 or 5°C. HPNTB stored for six months had lower ($p<0.05$) oiliness intensity than HPNTB at 5 or 25°C and PNTB at 50°C.

The smoothness intensity of HPNTB stored at 50°C increased significantly after 14 days of storage and remained unchanged for up to 84 days then decreased. HPNTB was not different from HPNTB at 25°C but was less smooth ($p<0.05$) than PNTB at 50°C. Storing HPNTB at 50°C had no effect on adhesiveness intensity. HPNTB was not different from HPNTB stored at 25°C or 5°C but was more adhesive than PNTB at 50°C.

HPNTB stored at 50°C had not change in spreadability. HPNTB had less spreadability intensity than HPNTB at 25°C and PNTB at 50°C.

Honey peanut butter containing 7% dry honey would not be significantly different from the control peanut butter for all the attributes. Honey peanut butter containing 7% dry honey had the same appearance and flavor characteristics of peanut butter containing no honey stored at room temperature for six months. HPNTB was less smooth and more adhesive than PNTB at 25°C. The roasted peanutty flavor of HPNTB was maintained for six months at room temperature (25°C).

Honey peanut butter stored at an accelerated temperature, 50°C, did not develop oxidized flavor but had darker color and high dark roasted flavor intensity. HPNTB was more adhesive and less smooth and less spreadable than PNTB.

2. Peanut Protein Film

Three studies were done in the area of development of peanut protein as edible film and coating. Materials used were: a) Roasted peanut (full fat) prepared from raw peanuts (Virginia type) roasted for 14 min at 163°C, skin and testae were removed by roller type blancher; b) partially defatted peanut flour (28% fat); e) defatted peanut flour (10% fat) processed by hexane extraction method; and, d) peanut protein concentrate (PC) prepared by modifying the classical method of alkaline extraction and acid precipitation from defatted peanut flour (2% fat).

a. Method of Film Formation

In the first study, comparison between two methods of film formation, surface film formation and deposition method was conducted. In the surface formation study, film forming solutions were prepared from roasted peanut (full fat), partially defatted peanut flour (10 and 28% fat) and protein concentrate. In deposition study, film forming solution was prepared only from protein concentrate since preliminary study showed that film could not be formed by solution from other raw materials. Protocol for preparing these films was given in last year's annual report. Color, puncture force, tensile strength (TS), percent elongation (E), water vapor permeability (WVP), and oxygen permeability (OP) were measured. The phenomena of film formation and film characteristics were observed. Effect of casting material [glass, plastic (polystyrene), aluminum foil, Teflon®, EKCO® non-stick bakeware], pH (5-11) and temperature (ambient temperature, 50, 60, 70, 80, 90°C) were studied in deposition method.

i. Surface film formation

The protein/lipid (P/L) ratio of full fat peanut milk (natural) was 0.56 (Table 11) which is very similar to the P/L of peanut milk produced by the others. The P/L ratio of raw materials were not considerably different from that of corresponding stock solution except for the protein concentrate. The pH of film forming solutions from roasted peanut (full fat) and partially defatted peanut flour (28% and 10% fat) ranged from 6.50 to 6.74 (Table 11) and we were able to form films from these solutions. Films could be formed in 20-30 min from roasted peanut (full fat), partially defatted peanut flour (28 and 10% fat) and protein concentrate solutions, but were fragile and thus difficult to harvest. However, a 2 hr-period allowed film to become thicker and stronger thus making harvesting possible. Films from the second, third and fourth harvesting were brown and darker than the first harvesting. This is attributed to the browning reaction from prolonged heating. Film from protein concentrate was found to be darker than films from other raw materials (Table 12). The surface of film from full fat peanut milk and 28% fat was very oily due to high fat content. As the P/L ratio increased the film surface was less oily. The water vapor permeability, oxygen permeability, tensile and elongation test of these films could not be determined due to their poor mechanical properties. Film from protein concentrate seemed to be stronger than films prepared from the other raw materials (Table 12).

ii. *Deposition method*

Preliminary study showed that at least 3% (w/w) protein is needed for film formation, film could be formed only from protein concentrate solution, is very brittle and fragile when plasticizer is not used. Films could be formed at pH 6 to 11, but not at pH 5 because pH 5 is very close to isoelectric point of peanut protein isolate (4.5). The color of film at pH 6 was yellower and lighter than that at pH 11. Films at low pH (pH 6) appeared opaque and dull because of many insoluble particles. At pH 11, film was yellow but dark and rather clear because of lesser insoluble particles. Dark color probably occurred due to alkalinity and heat reaction during film formation. Film formation from peanut protein (3%) occurred at 50-90°C and could not be formed at ambient temperature (>48 hr). At 9% protein content, film could be formed at room temperature after 48 hr but the surface of film was still moist. Aluminum foil laminated on glass dish was also tested; it was easier to peel the film off the aluminum foil than the glass dish directly. The smoothness of surface film depended on the smoothness of aluminum foil laminated on the glass surface. Teflon® pan was one of the molds we tested, it was very easy to peel off film without any considerable elongation or stretching; however, the side of the film adjacent to Teflon® surface had numerous tiny bubbles. A few bubbles occurred when glass or plastic dish was used. Film cast on ECKO® non-stick pan resulted in fewer bubbles and was easiest to remove. Thus, non-stick pan was considered as a casting mold in further studies. The WVP, OP, TS and E of film produced by deposition technique at pH 7.5 and 90°C were 11.80 $\pm$ 4.90 g.mm/(m^2.d.KPa) (film thickness was 0.1252 $\pm$ 0.0244 mm, at 50% RH, 37.8^{o}C), 1.53 $\pm$ 0.81 ml.:m/(m^2.d.KPa) (film thickness was 0.1201 $\pm$ 0.0204 mm, at 0% RH, 30°C), 3.15 MPa (film thickness was 0.1460 $\pm$ 0.0191 mm, at 50% RH, 25°C) and 126.67% (film thickness was 0.1460 $\pm$ 0.0191 mm, at 50% RH, 25°C), respectively.

Table 11. Proximate composition of raw materials and stock solutions

Material	%Moisture (wb)	%Protein (wb)	%Fat (wb)	P/L	pH
Raw material					
Full fat peanut	4.32	25.29	44.94	0.56	- -
Flour (28% fat)	7.67	35.44	25.86	1.37	--
Flour (10% fat)	2.44	46.69	10.09	4.64	--
Protein conc.	4.16	81.37	01.08	75.34	--
Stock solution					
Full fat peanut	85.40	3.88	8.04	0.48	6.63
Flour (28% fat)	86.15	5.92	4.20	1.41	6.74
Flour (10% fat)	87.74	5.50	1.74	3.16	6.65
Protein conc.	94.72	4.78	0.02	239	6.50

Table 12. Physical properties of film by surface formation (color and puncture force)

Raw material	Thickness (mm)	L*	a*	b*	Puncture force (N)
Full fat peanut	0.3575 $\pm$ 0.1071	59.37 $\pm$ 3.18	-1.08 $\pm$ 0.84	14.78 $\pm$ 4.12	0.25 $\pm$ 0.13
Flour (28% fat)	0.2555 $\pm$ 0.0320	66.31 $\pm$ 1.33	-1.99 $\pm$ 0.14 -	5.70 $\pm$ 1.68	0.15 $\pm$ 0.08
Flour (10% fat)	0.2193 $\pm$ 0.0406	63.02 $\pm$ 3.60	0.81 $\pm$ 0.45	10.73 $\pm$ 2.06	0.24 $\pm$ 0.12
Protein conc.	0.3760 $\pm$ 0.0438	42.84 $\pm$ 4.59	0.61 $\pm$ 0.54	6.76 $\pm$ 1.33	1.43 $\pm$ 0.45

b. Comparison of Properties

In the second study, the effects of temperature and pH on properties of edible peanut protein film (formed by deposition method) were investigated. Film forming solution of 3% (w/w) protein content and 4.76% (w/w) glycerine was prepared from peanut protein concentrate. Solution was adjusted to a desired pH (6.0, 7.5 or 9.0) and then heated to 70°C. Film was formed on a non-stick pan at three temperatures (70, 80 or 90°C). Moisture content, water activity, protein content, color (L*, a*, b*), film solubility, protein solubility, thickness, water vapor permeability (WVP), oxygen permeability (OP), tensile strength (TS) and percentage of elongation (%E) were measured. Results are summarized in Table 13.

Film formed at high temperature showed lower moisture content and water activity value. Temperature and pH didn't affect the amount of protein content, protein content was 49.07 $\pm$ 3.03% (db). Peanut protein film did not dissolve or break apart after 24 hr soaking in water. The solubility and % soluble protein of peanut protein film were affected by both pH and temperature. Total solubility and soluble protein decreased when temperature increased. The film solubility and soluble protein increased when pH increased, this result may be due to increase of protein solubility in film forming solution at high pH resulting in high solubility in dry film. The amount of glycerin used in peanut protein film may have resulted in higher solubility. Temperature and pH had significant effect on b* value, only pH had significant effect on L* and a*. Film color was darker and yellower when pH increased resulting in dark-yellow film at pH 9.

Film thickness ranged from 0.1053 to 0.2033 mm. Water vapor permeability (WVP) and oxygen permeability (OP) decreased from 37.47 to 10.35 g.mm/(m^2.d.KPa) and 15.40 to 2.13 ml.:m/(m^2.d.KPa), respectively as temperature increased from 70 to 90°C. TS and % E increased from 0.40 to 4.13 MPa and 73.7 to 146.5%, respectively as temperature increased from 70 to 90°C. pH had no significant effect on WVP, OP and TS, but had significant effect on % E. At pH 9 and 90°C, film had the smallest values of WVP and OP, and the highest TS. Compared to synthetic films, water vapor permeability of peanut protein film was lower than those from the other protein films, however, our test condition which was different from the others may have resulted in different values. Oxygen permeability of peanut film was slightly lower than soy protein and wheat film but much lower than synthetic film about 100 to 1000 times.

e. Sorption Isotherms

In the last study, sorption isotherms of peanut protein film were obtained and the effects of plasticizer on properties of peanut film were investigated. In this study film was formed by deposition method. Sorption isotherm of peanut protein film at pH 6.0, 7.5 and 9.0 were similar to the shape of the other edible films as shown in the literature. Moisture content of films formed at pH 7.5 and 9.0 increased sharply when relative humidity reached to 75%. This result showed a good agreement with isotherm of corn zein film. When compared with corn zein, peanut protein film had high equilibrium moisture content. The lower equilibrium moisture content in corn zein may be because of higher hydrophobicity of its amino acids. The percentage change of film thickness showed that thickness of film increased with increasing relative humidity. However, a decrease in thickness was observed at higher RH. This reduction is probably attributed to condensation of water vapor on the film surface which decreased the total solid in film and resulted in decreasing film thickness.

In the plasticizer study, film was formed by deposition method at pH 9.0 and 90 /C. Four types of plasticizers at 3 levels of each were chosen to study, glycerol (G), sorbitol (S), propylene glycol (PG) and polyethylene glycol (PEG) at 1.96, 3.38 and 4.76% of film forming solution (or film forming solution: plasticizer were 100:2, 100:3.5 and 100:5, respectively). Film thickness, water vapor permeability (WVP), oxygen permeability (OP), tensile strength (TS) and percentage of elongation (% E) were measured. In sorption study, film was formed at pH 6.0, 7.5, 9.0 at 80°C (using 3% protein content solution). Glycerin at 4.76% of film forming solution was used as plasticizer. Saturated salt solution were prepared to obtain 11, 33, 52, 75 and 93% RH, respectively. After drying for 16 hr film was cooled in desiccator for 15 min. Film was kept in desiccator for 7 days at 0% RH,

25°C and the thickness was measured; following which film samples were kept at 25/C and desired RH conditions. Thickness, a_w, and moisture content were determined after 9 days.

Preliminary study showed that film forming without plasticizer was very brittle and broke easily during peeling. It was found that a minimum concentration of glycerin 3.38% (w/w) in the film forming solution is needed. Properties of film with glycerin as plasticizer are shown in Table 14. WVP and OP values were not significantly affected by plasticizer level. Our results do not agree with the work reported in literature on other protein films where WVP and OP increased with increase in glycerin level. Plasticizer can retard or enhance moisture transmission depend on its concentration. However, the concentration of glycerin affected the mechanical properties TS and E because plasticizer can reduce the internal hydrogen bonding and increase the intermolecular spacing. Generally, TS decreased and E increased when glycerin level increased.

In conclusion, development of edible film from peanut protein concentrate using deposition method has much greater potential than by surface film formation method because of their uniformity. Film properties of peanut film formed by deposition method were affected by temperature and pH. Peanut protein film had high WVP but low in OP compared with synthetic film. Plasticizer was needed at least 3.38% (or 3:3.5 of protein:glycerin) of film forming solution for peanut protein film. Film was very hydrophilic and the thickness of film was affected by relative humidity.

Table 13. Properties of film by at different temperature and pH

Property	Temperature (/C)								
	70			80			90		
	pH			pH			pH		
	6.0	7.5	9.0	6.0	7.5	9.0	6.0	7.5	9.0
a_w	0.240	0.229	0.169	0.127	0.152	0.130	0.112	0.127	0.120
Moisture (%db)	36.60	32.54	28.65	24.17	24.19	23.14	14.50	12.72	17.14
Protein (%db)	48.04	50.19	44.99	49.36	49.74	47.45	49.54	51.42	50.87
Film sol. (%db)	41.16	55.27	48.09	33.72	42.68	41.52	32.17	39.86	40.02
Sol. protein(%)	9.98	22.61	20.09	6.21	12.47	16.99	5.43	10.37	12.37
WVP[1]	38.98	39.73	33.70	26.80	32.96	27.44	10.42	11.80	8.83
thickness (mm)	0.1911	0.1995	0.1692	0.1568	0.1684	0.1508	0.1099	0.1252	0.1006
OP[2]	16.80	13.12	16.29	9.45	10.10	7.66	3.69	1.53	1.16
thickness (mm)	0.1714	0.1793	0.1703	0.1548	0.1670	0.1594	0.1204	0.1204	0.1053
TS[3] (Mpa)	0.31	0.39	0.51	1.18	1.31	1.70	4.61	3.68	4.10
thickness (mm)	0.1892	0.1704	0.2033	0.1714	0.1703	0.1579	0.1227	0.1460	0.1459
E[3]	40.67	72.32	107.33	98.79	177.83	187.62	121.43	147.46	170.50
thickness (mm)	0.1892	0.1704	0.2033	0.1714	0.1703	0.1579	0.1227	0.1460	0.1459
L*	39.12	37.28	38.38	38.42	37.55	36.81	38.20	38.12	36.87
a*	1.38	1.1	0.98	1.34	1.10	0.84	1.13	0.90	0.85
b*	1.71	3.39	4.15	2.97	3.69	4.21	6.30	5.05	5.97

[1]g.mm/(m^2·d.KPa) at 50%RH, 37.8°C.
[2]ml.mm/(m^2·d.KPa) at 0%RH, 30°C.
[3]at 50%RH, 25°C.

Table 14. Properties of film at different level of glycerin

Properties	Concentration of Glycerin (%)*		
	1.96	**3.38**	**4.76**
WVP [g.mm/(m^2·d.KPa)][1]	9.03± 1.31[a]	10.64 ± 2.07 [a]	8.97± 4.11 [a]
thickness (mm)	0.1110± 0.0213	0.1105± 0.0174	0.1028± 0.0193
OP [ml. : m/(m^2·d.KPa)][2]	0.46±0.27 [a]	0.59± 0.32[a]	1.20± 0.93[a]
thickness (mm)	0.1248± 0.0277	0.1174± 0.0361	0.1068 ± 0.036
TS [MPa][3]	4.35± 0.1.21[ab]	5.14± 1.08[a]	4.10± 0.78[b]
thickness (mm)	0.1031± 0.0293	0.1097± 0.0287	0.1376± 0.0501
E [%][3]	105.00± 39.55[b]	124.50 ± 29.58[b]	163.93± 42.57[a]
thickness (mm)	0.1031± 0.0293	0.1097± 0.0287	0.1376 ± 0.0501

*of film forming solution.
The treatments with same letter within each row are not statistically different ($p \leq 0.05$).
[1]at 50%RH, 37.8°C, [2] at 0%RH, 30°C, [3] at 50%RH, 25°C.

3. Keeping Quality of Roasted Peanuts Coated with Edible Films

This study was conducted to quantify the degree of quality enhancement on roasted peanuts coated with either hydroxypropyl cellulose or zein and stored at 30/C. US Virginia extra large kernel shelled peanuts were dry roasted at 163/C for 30 min in an electric roaster. The peanuts were then cooled, blanched, and cleaned. The edible film solutions were made from corn zein (CZ) and hydroxypropyl cellulose (HPC) following the methods developed in our Center.

A peanut coater having a rotating stainless steel bowl with an adjustable inclination was fabricated by riveting two 40 cm dia. by 14 cm depth pans (one whose bottom was sawed or cut off) together. Batches of peanuts of 1.0 kg each were coated using the peanut coater at levels of 4, 7 and 9% of the initial batch weight of the product to be coated. Upon drying, the final weight of the coated raw material was recorded. Uncoated peanuts served as the control. The coated and uncoated peanuts were kept in an incubator at 30/C for a period of seven weeks. Each week, 60 g samples were collected in glass jars and stored at -18/C until they were analyzed for hexanal using gas chromatography. A two-thirds order model developed by Koelsch and her

$$[Hex] = \frac{1}{3}Kt^3 + C \qquad (1)$$

colleagues in 1991 was used for predicting the hexanal concentration in roasted peanuts. The following is the kinetic model with *K* as the rate constant,

$$\frac{d[Hex]}{dt} = (9K)^{\frac{1}{3}}[Hex]^{\frac{2}{3}} \qquad (2)$$

Nonlinear regression analyses using SAS® statistical package were performed to fit this model into the experimental data. The Koelsch rate constant was used to estimate the peanuts' keeping quality, *KQ* from the function:

$$KQ = -3.97\ln K + 0.21 \qquad (3)$$

The performance of CZ and HPC at three levels of application was compared. Coating caused a substantial delay in the production of hexanal, and consequently, the storage life of the product is expected to improve. However, the hexanal concentration of the coated peanuts do not appear to have any significant differences among the levels of application and type of the film solution. Table 15 summarizes the result of fitting the two-thirds order model (Eq. 2) of Koelsch into the various hexanal versus time data, and the keeping quality (Eq. 3). The rate constants show that coating peanuts with edible films slowed down the formation of hexanal to about one-tenth of the control. The keeping qualities of the coated peanuts were, therefore, enhanced by 1.60-1.83 times as much.

With increasing levels of application, the effectiveness of the film was supposed to improve, but it did not. This could be attributed to the mechanism of coating the film on the product. Since some of the edible coating was actually absorbed by the peanuts and did not form the thin film at the surface of the food, it was possible that interactions between the food and the coating affected the lipid oxidation reactions. The simplest way to avoid such interactions is to keep the film from being absorbed into the food. This could be achieved by decreasing the amount of film material or by improving the coating process. For example, drying at a slightly elevated temperature immediately after film application, so that the film is formed at the surface of the food.

In conclusion, coating peanuts delayed the production of hexanal, thus, enhancing the keeping quality of the food by 1.60-1.83 times as much. However, the levels of application had no significant effect on the storage life among the coated samples even with significant differences in the film thickness, oxygen permeance and water vapor permeance.

4. Peanut Drying Simulation Study

Peanuts contain excess moisture when harvested and unless the pods are rapidly dried to a safe moisture level, seed deterioration through fungal growth and aflatoxin production will rapidly set in. A number of variables can affect the rate of bulk peanut drying, which include the pod initial moisture, ambient dry bulb temperature (DBT) and wet bulb temperature (WBT), drying temperature and airflow. Drying time, moisture range within the bed of peanuts and energy usage of peanut dryers are in turn affected by the variables mentioned. The energy used in peanut drying are primarily due to the fan and the air heater.

Table 15. Estimation of keeping quality of roasted peanuts at 30/C storage

Edible film	Application level, %	Koelsch rate constant[a]		Keeping quality (KQ), wk	KQ/KQ_{ctrl}[b]
Control	0	0.04770	0.00605	12.3	1.00
HPC	4	0.004459	0.001992	21.7	1.77
	7	0.003687	0.001834	22.4	1.83
	9	0.005518	0.001698	20.8	1.70
CZ	4	0.004628	0.001587	21.5	1.75
	7	0.005103	0.002306	21.2	1.72
	9	0.007417	0.002047	19.7	1.60

[a] Treatments are significantly different from control at "= 0.05

[b] KQ/KQ_{ctrl} is the ratio of the KQ of the treatment over the KQ of the control.

The use of Hukill Method for analysis of bulk drying of grains such as peanuts can easily be done with a hand held calculator. Hence a peanut drying simulation program based on the Hukill Method was easily written in BASIC called PNUTDRY. PNUTDRY determines the thickness of a drying layer, the average moisture content of each layer, the overall bed depth and the average moisture content of the bed for a given pod initial moisture,

ambient temperature and humidity, drying temperature, airflow and drying time. Then the program determines the total airflow and total static load for a given dryer area which are then used in obtaining the size of the motor and the blower rotational speed. These information and the relevant data is then used in obtaining the fan, heater and total energy usage.

The PNUTDRY was used in the simulation analyses of a standard peanut drying wagon (8 ft by 14 ft floor area, 4.6 ft deep). The simulation program was run using approximate conditions used in the peanut drying data of Blankenship and Chew to test the adequacy of the simulation programs. The published data showed the effect of the pod initial moisture content and the type of dryer (single and double trailers) on the total drying time, total electricity used, total gas used and total energy cost. The data obtained from the simulation programs were also processed to fit these data.

After these simulations, the effects of several independent variables on drying time, bed moisture distribution, fan, heater and total energy usage in peanut drying were studied. The independent variables (including their levels) used in the simulation studies were the following: pod initial moisture (15, 20, 25 and 30% w.b.); ambient temperature (74, 77 and 80°F); ambient humidity (0.015, 0.017 and 0.018 lb water/lb dry air); drying temperature (87, 91 and 95°F); and airflow (55, 75 and 86 cfm/ft^2). Simulations were carried out until the final average moisture content of the bed of peanuts is about 11% (w.b.).

Data obtained at air flows of 55 and 75 cfm/ft^2 and humidity conditions of 0.0168 and 0.0185 lb water/lb dry air were within the spread of data reported by Blankenship and Chew in 1979. These shows that the simulation model can adequately predict the drying times in peanut dryers. The drying times at both airflows with higher humidity seems to better represent the drying conditions of the published data. Hence in the succeeding simulations, only these two conditions were used for comparison of the energy usage in peanut dryers.

The drying time increased with increasing pod initial moisture due to the greater amount of water that needs to be evaporated. The ambient temperature had an insignificant effect on the drying time of peanuts because the drying temperature is controlled at 95°F independent of the prevailing ambient conditions. The drying time of peanuts increased with increasing ambient humidity. The drying time was affected by the ambient humidity since this influences the equilibrium moisture content (EMC) and the drying rate of peanuts and the properties of moist air which all affect the bulk drying of peanuts. The drying time increased with decreasing airflow by affecting the rate of drying. The amount of increase in drying time was much higher as the airflow decreased from 75 to 55 cfm/ft^2 than from 86 to 75 cfm/ft^2. The drying time of peanuts increased with decreasing drying temperature. Drying temperature also influences the EMC and the drying rate of peanuts and the properties of moist air which all affect the bulk drying of peanuts.

The bottom layer moisture was not affected by ambient temperature but decreased with increasing pod initial moisture. The moisture content in the bottom layers was also much nearer to the equilibrium moisture content for pods with higher initial moisture. The top layer moisture was slightly affected by ambient temperature and increased with increasing pod initial moisture. The bottom layer decreased with increasing pod initial moisture because of longer drying time exposure. However, the top layer moisture increased with increasing pod initial moisture because the drying time was not long enough to overcome the moisture gradient. The top and bottom layer moisture decreased with decreasing ambient humidity. Moisture content in the bottom layers was lower for low humidity drying because of its lower EMC. The much lower moisture in the top layer for pod with higher ambient humidity was due to the increase in its EMC which therefore decreased the drying rate. The bottom layer moisture decreased with decreasing airflow. However, the top layer moisture increased with decreasing airflow. The lower moisture content of the bottom layer with lower airflow was due to the longer drying time exposure. But the higher moisture of the top layer with lower airflow was due to the decrease in the bulk drying rate. The bottom layer moisture decreased with increasing drying temperature. The top layer moisture increased with increasing drying temperature. The bottom layer moisture were lower for high drying temperature due to

its lower EMC. However, the top layer moisture were lower for lower drying temperature because of its longer drying time exposure thereby overcoming the effect of moisture gradient.

The ambient temperature had no significant effect on the fan energy usage of peanut dryers. The ambient temperature has no effect on drying time and so the fan energy usage was also not affected since it is only dependent on drying time. However, the heater and the total energy usage increased with increasing pod initial moisture and decreasing ambient temperature. When the pods had higher initial moisture more heat is needed to evaporate the higher moisture load. When the ambient temperature decreases there would be more heat energy needed to attain the required drying temperature. The total energy usage also increased consequently. The fan energy usage were only 5.5 and 7.5% of the total energy used in peanut drying at ambient temperature of 74 and 80°F, respectively. The fan, heater and total energy usage increased at increasing ambient humidity. The fan energy usage was only about 6.3% of the total energy use in peanut drying at all humidity levels. The increase of fan, heater and total energy usage with increasing ambient humidity were mainly due to increase in the drying time. The fan, heater and total energy usage increased at increasing airflow. The fan energy usage were only 5.1 and 8.0% of the total energy used in peanut drying at airflow of 55 and 86 cfm/ft^2 ^{o}F, respectively. The fan energy usage increased with increasing airflow since the size of the motor will increase accordingly. The heat energy usage increased with increasing airflow since more volume of air needed to be heated to the required drying temperature. Consequently the total energy usage increased due to the increased in fan and heat energy usage.

The fan energy usage increased with decrease in drying temperature. However, the heater and total energy usage increased with increasing drying temperature. The fan energy usage were only 6.3 and 10.8% of the total energy used in peanut drying at drying temperatures of 95 and 87°F, respectively. The fan energy usage increased with decreasing drying temperature because of longer drying time involved. The heat and total energy usage increased with increasing drying temperature because more fuel is needed to attain the required drying temperature.

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In conclusion, the simulation program (PNUTDRY) developed at our center was found to be valid in predicting the drying conditions and can be used in the design of peanut dryers of desired capacity with optimum drying conditions. Drying temperature had the most significant effect on the drying time followed by airflow and then ambient humidity. The ambient temperature within the range studied had no effect on drying time and also bed moisture range. Airflow had the major effect on moisture content of top layer. The fan energy usage was not affected by the drying temperature and airflow while the heater energy usage was affected by all the variables studied. Based on the energy usage and drying time information, drying the peanuts at 95/F of drying temperature, 60 cfm/ft^2 air flow and 76/F wet bulb temperature was found to produce optimum drying conditions with a drying time of 15 h for 1600 lb wet pods at 25% wet basis.

5. <u>Bacterial Degradation of Aflatoxins</u>

a. Role of Divalent Cations and Chelators

Experiments were done in an effort identify the biochemical mechanism by which *Flavobacterium aurantiacum* degrades aflatoxin B_1 (AFB1). The first set of experiments were conducted to establish the influence of divalent cations and chelators on AFB1 degradation by *F. aurantiacum*. The divalent cations studied so far include Ca^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, and Mn^{2+}. The effect of chelators, EDTA and 1,10-phenanthroline (OPT), in the presence of divalent cations, on AFB1 removal by *F. aurantiacum* have also been studied. Controls containing aflatoxin without the addition of *F. aurantiacum*, aflatoxin and the divalent cations, aflatoxin and chelator, and aflatoxin and divalent cation and chelator, were used accordingly to determine any chemical (non-biological) degradation of AFB1.

HPLC was used to quantify aflatoxin degradation by viable cultures of *F. aurantiacum*. Maximum degradation was obtained when *F. aurantiacum* cells were incubated for 48 h in the presence of AFB1. The addition of 0.1, 1, and 10 mM divalent Ca slightly increased aflatoxin removal after 16 and 24 h but the increase was only significantly different from controls after 48 h ($p < 0.05$). Similar results were obtained with 0.1, 1, and 10 mM Mg. Control samples incubated with aflatoxin in phosphate buffer alone (without cells) and controls containing Ca or Mg and aflatoxin in phosphate buffer (without cells) did not result in significant removal of aflatoxin (< 5%) after 48 h. Significantly higher aflatoxin removal was seen after 48 h incubation with the addition of Mg.

The enhanced removal of AFB1 caused by the addition of divalent cations (Ca and Mg) could be attributed to the fact that these divalent cations are important cofactors of various dehydrogenases and decarboxylases in glycolysis and in the TCA cycle. Since reduction and decarboxylation are means by which aflatoxin is degraded, these cofactors may be enhancing the enzyme or enzyme system responsible for aflatoxin degradation and keeping the system in an active form for a longer period of time. Enhanced degradation could be attributed to AFB1 being the only carbon source for *F. aurantiacum*. In this case, as the C source gets depleted, the divalent cations may be complexing with either ATP or the enzyme system to enhance the metabolic rate of the cell thus enhancing degradation.

Addition of EDTA to the whole cell preparations increased aflatoxin removal after 24 h without significantly increasing removal of AFB1 at 16 or 48 h incubation. Similarly, addition of 0.1 and 1.0 mM OPT resulted in significantly higher rates of removal after 24 h incubation. However, a decrease in removal was observed after 16 and 24 h incubation with test solutions containing of 10 mM OPT. The increase in aflatoxin removal with added EDTA and OPT (0.1 and 1 mM) could be due to the binding of inhibitors (possibly divalent cations other than Mg and Ca), interaction with AFB1 in some unknown manner, or enhancement of the metabolism of the cell to degrade AFB1 more rapidly. The decrease in aflatoxin removal observed when 10 mM OPT was present could be due to an inhibitory effect caused by the high concentration of the chelator blocking some metabolic pathway of the cell or binding of the essential divalent cations.

Addition of divalent cations in the presence of chelators resulted in no significant difference in aflatoxin removal for divalent Ca in the presence of EDTA. However, the addition of Ca significantly lowered rates of removal in the presence of OPT, with the highest removal occuring with 10 mM Ca in the presence of 1 mM OPT and decreased degradation with 1 mM and 0.1 mM Ca in the presence of OPT. Addition of divalent Mg resulted in significantly less removal of AFB1 in the presence of both EDTA and OPT, as compared to the control. This suggests that divalent Mg is strongly bound by the chelators and that is possibly the important cofactor involved in aflatoxin removal by the bacterium. Divalent Ca is bound more strongly by OPT and therefore may be made unavailable to the cell, resulting in less AFB1 degradation than was observed when divalent Ca alone was added to the cells containing aflatoxin.

These results demonstrate that the divalent cations Ca and Mg stimulate AFB1 removal by *F. aurantiacum* and that binding of these cations by chelators make them unavailable to the cell and decreases aflatoxin removal. Based on our results, it is likely that divalent Mg is the cation with the higher stimulatory effect. Divalent Mg is known to be an important cofactor and stimulator of the pyruvate dehydrogenase system in the glycolytic pathway as well as pyruvate decarboxylase. Divalent Mg is also known to bind ATP and activate the substrate, transfer acyl groups, phosphate groups (kinases) and increase reaction rates. From earlier work completed under this project and from the results of this study, the probable mechanism of AFB1 degradation by *F. aurantiacum* is via decarboxylation of the coumarin ring to give detoxified products that are water-soluble and chloroform-soluble with the release of gaseous CO_2.

b. Role of Trace Metal Cations on Aflatoxin Removal

Research was also undertaken to determine the effect of trace metal cations on aflatoxin removal. The trace metal ions studied so far include divalent zinc, divalent Mn, divalent Cd and divalent Co. Divalent Zn is a

cofactor of alcohol dehydrogenase and is also important in DNA polymerase and carbonic anhydrases. However, Zn is also an inhibitor of glucose-6-phosphate dehydrogenase. No significant difference in aflatoxin removal was observed after incubation with 0.1 mM Zn. However, significantly decreased aflatoxin removal was observed using 1 mM and 10 mM Zn after 4 and 24 h as compared to control without Zn. The addition of 1 mM EDTA or OPT with 0.1, 1 and 10 mM Zn was studied to determine if binding of Zn by the chelators would remove the inhibitory effect by Zn and thus increase aflatoxin removal. OPT is known to have a high affinity for divalent Zn, copper (Cu^{2+}), ferrous iron (Fe^{2+}) and ferric iron. Zinc in the presence of EDTA resulted in no significant difference in aflatoxin removal compared to when Zn alone was added to the cells containing aflatoxin. This suggests that EDTA did not bind Zn well enough to make it unavailable to the cell. In contrast, OPT bound Zn and removed the inhibitory effect by 1 mM Zn. Although the addition of 10 mM Zn in the presence of OPT resulted in a higher percentage aflatoxin removal than when 10 mM Zn alone was added, the percentage aflatoxin removal was still significantly lower compared to the control cells containing only aflatoxin. Zinc could be binding the enzyme or altering the enzyme system by causinge a conformational change in the enzyme(s), resulting in an inactivation of the enzyme or a form of the enzyme(s) with reduced aflatoxin removal.

Experiments with divalent Mn were conducted because Mn is an important cofactor in lignin peroxidases of fungal systems and in carbofuran hydrolase of *Achromobacter* species. Another reason for studying several cations was to determine the specificity of inhibition or stimulation. The addition of 0.1 mM Mn did not result in a significant difference in aflatoxin removal as compared to the control containing only AFB1. However, 1 and 10 mM Mn resulted in decreased removal after 4 and 24 h. These higher concentrations may be toxic to the cell itself or the genes of the aflatoxin degradation system are being repressed or the enzyme protein is being converted to an inactive or lower affinity form for aflatoxin removal at higher concentrations of Mn. Binding of Mn by EDTA and OPT (except for 10 mM Mn by OPT) resulted in increased removal as compared to when Mn alone was added to the cells containing aflatoxin.

The effects of Cd was studied to determine if a sulfhydryl group was involved in aflatoxin removal. Cadmium is toxic to most cells as it binds essential sulfhydryl groups making them unavailable for activity as well as denaturing proteins. Microorganisms tolerate Cd by binding toxic cations to long chain polymers of polyphosphate. They also gain resistance to Cd by harboring plasmids that can convert the toxic cations to a non-toxic form. Decreased aflatoxin removal was observed using 1 mM and 10 mM Cd as compared to cells containing AFB1 alone. EDTA or OPT did not counter the inhibition of aflatoxin removal by addition of 1 and 10 mM Cd. These concentrations could be toxic to the cell by blocking the metabolism of the cell and decreasing aflatoxin removal. This toxic effect could have been overcome over a time of 48 h in order that the bacteria survive. Divalent Co caused no significant difference in aflatoxin removal as compared to cells containing aflatoxin alone. Therefore, no further studies were carried out with Co.

The effects of divalent cations and chelators on aflatoxin removal has helped us gain an understanding of the enzyme system/s involved in aflatoxin removal. This study has helped us postulate a dehydrogenase (reductase) system and a decarboxylase system involved in AFB1 removal. Further work on the cellular fractions will be necessary to determine to effect of these divalent cations on crude enzyme preparations and determination of their effect directly on the crude preparations.

TRAINING ACCOMPLISHMENTS

Dr. Penkwan Chompreeda and Vichai Harathathanasan a Kasetsart University organised a Workshop on training trainers on the topic of "Quality Evaluation and Utilization of Food Legumes" which was held April 22 to May 24, 1996. The workshop was attended by 13 participants from countries: Tanzania-East Africa (2), Bhutan (2), Sierra Leone (2), Loa P.D.R. (2), Republic of Guinea (1) and Thailand (4). All participants were government officers whose work included legume utilization. Proceedings were distributed at the workshop. Many examples given during the training were peanut products developed from the Peanut CRSP project. Cooperation with the Department of Technical and Economic Cooperation, Office of Prime Minister under Thai-AID project facilitated the training course. This training course, which will be conducted annually, will have

a large impact on processing legumes as a whole and the nutritional well-being of consumers throughout Southeast Asia as well as other developing countries in the world in future years.

As part of technology transfer activities of the project, Thai Peanut CRSP researchers continued to cooperate with home economists from the Department of Agriculture Extension, Ministry of Agriculture to transfer technology of ground roasted peanut, hot sand roasted peanut and coated peanut processing to housewives in the Kud-Jub District, Udorn-thanee Province. Housewives were very receptive and, at the present, they produce and market these products in that area. This site has served as a model for some other villagers, especially housewives in the Northeastern Thailand and in Laos P.D.R., to develop processing technologies for other agricultural products.

A 2-day Workshop on Peanut Processing was held at the Institute of Food Science and Technology (UPLB). Trainers were Dr. Ricardo R. del Rosario and Ms. Olivia M. del Rosario. There were eight participants.

A Seminar-Workshop on Aflatoxin Control for the Food Industry, organized by Dr. Virgilio V. Garcia, was held at the Institute of Food Science and Technology, University of the Philippines at Los Banos. The following report summarizing the 2-day program was prepared by Dr. Garcia.

The availability of quality processed foods remains a continuing concern inspite of the adoption of modern food technologies. As early as 1988, food scientists from U. P. Los Banos called the attention of the Bureauof Food and Drug (BFAD) to the presence of high levels of aflatoxin in raw peanuts and peanut products. In 1993, BFAD recalled several brands of peanut butter from the market because they were found to contain more than 20 ppm aflatoxin. This result in decreased sales which affected peanut processors. To cope with the problem, the food industry (producers and manufacturers) requested a training program on aflatoxin control and analysis, including sampling methods and quality and safety procedures. In addition, an information campaign on the health aspects of mycotoxins in foods was targeted toward consumers.

A Seminar/Workshop on Aflatoxin Control for the Food Industry was held May 13-14, 1996 at the Institute of Food Science and Technology (IFST), U. P. Los Banos. The undertaking was co-sponsored by IFST (UPLB), USAID Peanut Collaborative Research Support Program (CRSP) and the Center for Food Safety and Quality Enhancement, University of Georgia, in cooperation with the Bureau of Food and Drugs, Department of Health, and the Food and Nutrition Research Institute, Department of Science and Technology.

The objectives of the seminar workshop were to update knowledge on aflatoxin research and assay methodologies and to thresh out and harmonize analyses related problems, tapping the expertise of local and foreign experts. The Seminar/Workshop primarily addressed the peanut processing industry, peanut growers and peanut traders. Of the 20 participants in the Workshop, 13 were from the food industry (entrepreneurs, Research and Development, and Quality Assurance managers and quality control personnel), 1 was from a nongovernment agency from Mindanao and 6 were from the academe (1 associate professor from the Institute of Chemistry, 1 instructor from U. P. Visayas and 4 university research associates from IFST.

The program consisted of presentations on analysis of aflatoxigenic fungi and an update on aflatoxin research and development activities, as well as an update on rapid tests. Hands-on analysis using AOAC methods was conducted by the BFAD personnel in an afternoon session. Peanut-CRSP researchers who presented lectures were Drs. Ricardo R. del Rosario, Reynaldo C. Mabesa and Robert E. Brackett. Dr. Brackett also gave a demonstration on rapid methods of analysis."

Mr. Panuwat Suppakul, Ms. Wimolsiri Thanavisuthra and Ms. Supang Reungchay completed their M.S. degrees at the Department of Product Development, Kasetsart Univerity. Ms. Wimolsiri Thanavisuthra resumed her teaching position at the Faculty of Biotechnology, Rungsit University (private university) in Bangkok. Ms. Supang Reungchay has taken a position as lecturer at the Department of Food Technology, Faculty of Science,

Ramkamheang University in Bangkok. Mr. Panuwat Suppakul is preparing himself to enter the Ph.D. program abroad.

Dr. Hathairat Uapithak from Kasetsart University and Dr. Lutgarda S. Palomar from VISCA received 4 weeks of training in sensory evaluation methodology at the University of Georgia with Dr. Anna Resurreccion. They conducted a descriptive sensory analysis study on shelf stability of honey peanut butter.

Dr. Lemuel M. Diamante from VISCA spent 6 months in the laboratory of Dr. Manjeet Chinnan. He learned how to use 2-D and 3-D graphics computer software to make drawings for designing food processing equipment. He prepared two manuscripts reporting results of research conducted at the University of Georgia and developed design parameters to construct a peanut drying in the Philippines.

Mr. Witoon Prinyawiwatkul (Thai), Mr. Anuvat Jangchud (Thai) and Ms. Doris D'Souza are pursuing Ph.D. degrees at the University of Georgia. Mr. Prinyawiwatkul receives 50% of his financial support from USAID Bean/Cowpea CRSP.

Mr. Augustin Antonio T. Ramos (Filipino) completed his M.S. degree program at the University of Georgia and returned to the Philippines.

Mr. Zhang Zhong (China) is pursuing an M.S. degree at the University of Georgia.

PRESENTATIONS AND CONFERENCE PARTICIPATION

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Ramos, V., A. A. T., M. S. Chinnan, M. C. Erickson and V M. Balasubramaniam. 1996. Lipid oxidation in roasted peanuts coated with edible films. Abstr. Annu. Meet. Inst. Food Technol., New Orleans, LA, June 22-26.

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INTERNATIONAL TRAVEL

Dr. Manjeet Chinnan traveled to the Philippines to meet with researchers at ViSCA and the University of the Philippines at Diliman to develop a project proposal and to finalize plans for Dr. Diamante (ViSCA) to visit UGA.

Dr. Anna Resurreccion traveled to The Philippines to discuss research progress with co-investigators from the University of The Philippines at Los Banos and ViSCA at Baybay, Leyte. She also visited the Food Development Center and the Asian Social Institute in Metro-Manila.

Dr. Larry Beuchat traveled to the Philippines and Thailand for the purpose of discussing final-year research activities and plans for training courses and workshops.

Dr. Robert Brackett traveled to The Philippines to present lectures at the workshop on “Aflatoxin Control for the Food Industry” held at the University of the Philippines at Los Banos.

Dr. Virgilio Garcia (UPLB), Dr. Lutgarda Palomar (ViSCA) and Dr. Hathairat Uapithak (KU) traveled to the U. S. to attend the annual meeting of the Institute of Food Technologists held in New Orleans.

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Prinyawiwatkul, W., K. H. McWatters, L. R. Beuchat and R. D. Phillips. Physico-chemical and sensory properties of chicken nuggets extended with fermented cowpea and peanut flours. J. Agric. Food Chem. (Submitted).

Prinyawiwatkul, W., K. H. McWatters, L. R. Beuchat and R. D. Phillips. Optimizing consumer acceptability of sensory qualities of chicken nuggets extended with fermented cowpea and peanut flours. J. Food Sci. (Submitted).

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GA/PH/C

Postharvest Handling Systems For The Small Peanut Producers

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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.

Need for improved postharvest operations exist not only in the Caribbean region but also in the Southeast Asian countries, such as Thailand and the Philippines, and other peanut growing regions of the developing nation. Emphasis continued in the Caribbean region in the current fiscal year, whereas collaboration in Thailand which was initiated in 1993-1994 continued through June 1995. The collaboration undertaken at Khon Kaen University in conjunction with the peanut utilization project (GA/FT/TP) at Kasetsart University, Bangkok, Thailand, for an improved system of postharvest operations and providing linkage with the production and utilization aspects of peanuts. Details of the Thai collaboration component is described in the GA/FT/TP report for this year.

Overall due to a very reduced level of funding for the postharvest activities in general, and those for the Caribbean region in particular, project activities with CARDI were very limited in the current fiscal year.

ACCOMPLISHMENTS

Belize
The main emphasis was on the socioeconomic study. The questionnaire was finalized based on the instrument developed for the Jamaica study, and the field data was collected during this year. Efforts continued for activities in the area of maintaining post harvest quality and reducing post production cost through improved post harvest handling operations including harvesting, threshing/stripping shelling and storage.

Jamaica
Main emphasis was on conducting training sessions for consumer taste panels and aflatoxin monitoring in peanut and peanut products. Dr. Margaret Hinds traveled to Jamaica for one week to conduct the training sessions. CARDI has been working very closely with UFMC (United Farmers Multi-purpose Cooperative), and

has made significant progress in training of personnel and utilization of postharvest handling equipment developed or obtained through the project.

US Institution - University of Georgia
The main emphasis was coordination of activities in Jamaica as well as in Belize. These activities are described under respective host country accomplishments. The work done by two graduate students (Ramos and Jangchud) applicable to the U. S. food industry has been described in detail in Dr. Larry R. Beuchat's project (GA/FT/TP).

IMPACTS

Identification, modification and/or adaptation of postharvest equipment and operations to improve quality of peanuts. Medium and small scale systems such as mechanical threshers, shellers motor driven and pedal powered), mechanical dryers (stationary and portable) are available to the farmers associated with cooperatives (typically in Belize) or individual farmers (typically in Jamaica) and also to the members of UFMC cooperative in Jamaica. The design of these equipment is also available so that these can be fabricated locally.

Improving quality of peanut products in host countries by working with local processors. Efforts have been made to work closely with the processors to assist in improving their peanut products. For example, in Belize these are Piache, Triple-F, and Burns; in Jamaica these are Pioneer Chocolate, and Blakey's.

Training and professional development. This is an integral part of the project both in Belize and Jamaica by working closely with BFAC and Ministry of Agricultural/Rural Agricultural Development Association, respectively.

Developing products and processes to benefit the US consumer and US food industry. Development of a prototype peanut based beverage, and edible films based on peanut proteins.. Additional funding and close cooperation with a US company should allow us to bring this project closer to commercialization.

GOAL

To develop a global model for evaluating the acceptability and profitability of peanut postharvest system(s) for Caribbean countries, in particular, and developing countries, in general, by developing and/or adapting technologies for the available economic and natural resources and by incorporating climate, socioeconomic and existing infrastructure for marketing and consumer acceptance factors.

OBJECTIVES

The proposed research is focused on attainable goals during the five year period beginning July 1, 1990. Specific objectives are:

1. Identify the segments of the postharvest handling system, and various constraints, and develop a qualitative relationship(s) among the segments.
2. Evaluate the economic value of each postharvest handling operation or segment identified.
3. Identify existing technologies for adaptation or modification.
4. Evaluate the technologies identified for functionality and economic value.
5. Evaluate the anticipated change in quality loss.
6. Determine the physical properties of peanuts and associated materials.
7. Identify the input and output parameters of all the components of the global model, and build the model.
8. Develop alternative scenarios of integrated systems.

ORGANIZATION

U. S. Lead Institution: University of Georgia
Center for Food Safety and Quality Enhancement, Department of Food Science and Technology, Griffin
Dr. Manjeet S. Chinnan, Principal Investigator
Mr. Glen D. Farrell, Research Coordinator I
Dr. P. Mallikarjunan, Postdoctoral Research Engineer
Dr. V. M. Balasubramaniam, Postdoctoral Research Engineer

Host Country Institutions:
CARDI (Caribbean Agricultural Research and Development Institute)

Belize
Mr. A. K. Sinha, Principal Investigator
Mr. Neville C. McAndrew, Agronomist
Mr. Martin Lindo, Technical Assistant
Mr. Bert O. Enriquez, Manager, BFAC (Belize Federation of Agricultural Cooperative).
Mr. Javier Garcia, Agronomist, BFAC.
Mr. Cristobal Teck, Extension Officer (Ministry of Agriculture)

Jamaica
Dr. Joe Lindsay, Principal Investigator
Dr. Janice Reid, Country Representative
Mr. Morris Taylor, Field Assistant
Mr. Neville Wright, Field Assistant
Mrs. C. Wilson, Extension Officer, RADA/MOA (Rural Agricultural Development Authority/Ministry Ministry of Agriculture)

ACCOMPLISHMENTS IN DETAIL

RESEARCH

A. Belize
Several constraints to peanut production in Belize have been recognized. Among these is the high cost of production primarily as a result of the extensive use of manual labor in the production process. Except for land preparation, all other operations from planting to harvesting and threshing are usually performed manually. Under Peanut CRSP activities in collaboration with the University of Georgia successfully designed and tested an engine driven thresher in 1990-91. Two (2) threshers, one engine driven and the other PTO driven, were later locally fabricated and are currently available for use by peanut growers. In 1992-93 a tractor mounted peanut lifter, specifically designed for use on heavy clay soils was locally fabricated and was tested in heavy and lighter soils. Mechanical lifting and threshing operations are expected to substantially reduce the cost of peanut production.

Dryer
Based on the successful introduction of artificial dryer at the San Antonio Cooperative, another Cooperative made request for construction of dryer at their cooperative site. A design was provided to the cooperative with the cost of materials and equipment. CARDI assisted during the construction of dryer by the regular visits to the site and provide appropriate recommendations as needed. The construction of the dryer was completed and a test run was done using peanut. It was found working very efficiently and economically. The cooperative members were drying their peanut and corn whenever it was raining. The members would be expanding production acreage under peanut.

A portable dryer has been locally fabricated based on the design developed by the University of Georgia. There were some modifications made on the size of the dryer and space between the dryer and the blower. The dryer could dry about 2,500 lb. of peanut and about 4,500 lb. of corn, soybean and rice. A number of test run was conducted for dying of various grains and was found very efficient and economical.

In 1993-94 kerosene burner was replaced by locally fabricated butane gas burners which provided cleaner hot air for drying and is safer for drying seed. Based on the proto-type developed a complete design was prepared and then fabrication was made. It was found very successful and provided cleaner product. Now four dryers are using this type of burner unit.

Socio-economic Study

A total of 51 farmers were selected for participating in this study. This represented about 75 percent of the peanut farmers in Belize. These peanut farmers were primarily located in the Cayo District (94 percent). About 55 percent of the farmers were in the 30-49 years age group and about 29 percent of them were in more than 50 years of age group. Just over 64 percent of the farmers completed primary school. The data also indicate that about 18 percent of the farmers did not attend school. There were 36 farmers (71 percent) of the total farmers use lease land from the Government and about 8 farmers (16 percent) were using cooperative land.

All farmers uproot their crop and strip their peanut by hand. The farmers were primarily dependent on sun drying for the harvested peanut. More than 70 percent of the farmers stored their peanut at their home and about 21 percent of them stored at the cooperative storage. About 98 percent of farmers selected their good quality peanut during cleaning process and at that time immature and moldy peanut were separated. About 88 percent of farmers stored their mature and dried peanut in coccus bags. About 48 percent of farmers reported drying was their biggest post-harvest problem while about 19 percent reported getting peanut out of the field was the biggest problem.

About 49 percent of farmers sold their produce to the cooperative, 33 percent to processor and about 20 percent to the local middlemen. About 59 percent of farmers were earning more income from other crops and about 39 percent of farmers earned most of their income from peanut. There was only one farmer who was earning more than BZ $ 20,000 (US $ 10,000). About 65 percent of the farmers earned in the range of BZ $ 1,000 - 4,999.

About 92 percent of farmers felt that the capital cost of equipment in postharvest operation hinders in their acceptance. About 72 percent of the farmers were willing to share the cost and use of equipment with other farmers. About 94 percent of the farmers were willing to contract for a fee with an organization (or a person) with equipment to facilitate production and postharvest handling of peanut.

Aflatoxin

Aflatoxin level was monitored in exporting peanut. The samples were checked and all showed aflatoxin levels below tolerance level (20 ppb).

B. Jamaica

United Farmers Multi-purpose Cooperative (UFMC). CARDI has been working closely with the UFMC. Although UFMC is still in its infancy, it has made steady progress since it was initiated about three years ago. CARDI has been assisting the cooperative in terms of inputs on the production of peanuts as well as postharvest handling, storage and marketing of peanuts.

Two peanut threshers operated by CARDI have been used by the cooperative members. The use of the threshers was demonstrated to three groups of farmers (about 50 persons) in several locations including Grosmond and New Hampton areas of St. Elizabeth. Subsequent to the demonstrations the threshers have been

used on several farms during the Spring 1996 crop. The motorized and hand operated threshers are now managed by the cooperative.

In the area of marketing, the cooperative members produce the peanuts and the Co-op collects and markets in bulk. The cooperative continues to receive assistance in the training of members in peanut processing. Current activities include solar roasting of peanuts and making peanut candies. These products are sold through the schools in the area.

Summary of Activities during Dr. Hinds visit. Objectives of the visit were:

1. To train farmers, small-scale processors and technical staff to test peanuts for the presence of aflatoxin.

2. 'To visit small-scale peanut processors to advise them on methods to improve the quality of their products.

Objective 1: Two Workshops (one in Kingston and one in St. Elizabeth) were held to train farmers, small-scale processors and technical staff to test peanuts for the presence of aflatoxin. A total of 32 persons were trained to use the AflaScreen Test (VICAM, Watertown, MA, USA), a mini-column analytical test which was developed by researchers at Texas A&M University. Each participant was given a copy of a 'Laboratory Manual on Testing Peanuts for Aflatoxin' which was prepared at the Center for Food Safety and Quality Enhancement, The University of Georgia, and which contained the experimental procedures in a format appropriate for persons with no previous laboratory experience. Methods to improve the protocol "or screening local peanuts for aflatoxin were discussed with the Senior Food Storage Scientist, Food Storage and Prevention of Infestation Division.

Objective 2: Meetings were held with small-scale processors from RADA (Kingston and Sandy Bay Districts), Lime Tree Garden Village Peanut Cultivation & Processing project (St. Ann), and the United Farmers Multipurpose Co-operative (St. Elizabeth). Discussions focused on methods to improve the quality of existing products, and technologies for introduction of new products. The chief products presently manufactured are dry-roasted peanuts, peanut drops, peanut cookies, and a corn-soya-peanut cereal. Information on processing protocols for a variety of alternative peanut products were left with Dr. Lindsay for distribution to the peanut processors.

C. U. S. Institution - University of Georgia

The main emphasis was coordination of activities in Jamaica as well as in Belize. These activities are described under respective host country accomplishments. The work done by Dr. Hinds (Post-doc) and graduate students (Ramos and Jangchud) applicable to the U. S. food industry has been described in detail in Dr. Larry R. Beuchat's project (GA/FT/TP).

TRAINING

CARDI-Jamaica Personnel: Dr. Margaret Hinds who was in the past associated with the project and is now on the faculty at North Carolina A&T University traveled to Jamaica to provide training on various aspects of postharvest handling, product development and quality evaluation. Details of her travel is given in the section above. Morris Taylor, Dr. Joseph Lindsay and Carol Wilson offered technical assistance and training as needed to peanut growers in Jamaica.

CARDI-Belize Personnel: Members of several cooperatives associated with BFAC received lectures on production and postharvest handling of peanuts. A number of field days were organized jointly with BFAC. CARDI Belize continued to provide training to Extension Officer of the Ministry of Agriculture, one Agronomist and one Extension Officer of the Belize Federation of Agricultural Cooperative (BFAC).

INTERNATIONAL TRAVEL

Dr. Margaret J. Hinds traveled to Jamaica from June 2 to 8, 1996 to hold a training session for aflatoxin monitoring, and advising local processors to improve quality of their peanut products.

Niger InterCRSP Project

Alabama A&M University and The University of Georgia
Institut National Recherches Agricoles Niger

Chandra Reddy, AAMU and David Cummins, UGA, and Issaka Mahamane, INRAN
Co-Principal Investigators

INTRODUCTION

A number of CRSPs have been active in Niger for several years, as well as in other West African countries. The CRSP Council made up of all CRSP Directors (presently nine programs) began discussing the value and potential of an organized cooperative program of research and outreach. In 1991, a group of the Directors met with John Mitchell, the Agricultural Officer from USAID/Niger, at a Sorghum and Millet CRSP meeting in Corpus Christi, Texas. We discussed the feasibility of an InterCRSP project that would support a cooperative effort of several CRSPs in Niger. A plan was developed for funding, but for various reasons including reduction of USAID/Niger budgets, the program was not implemented. The CRSPs were again approached by the USAID/Niger Agricultural Officer, Curt Nissley in late 1994 to reconsider an InterCRSP activity. Specifically, the Sorghum/Millet. Soil Management, and Peanut CRSPs were cited for collaboration. A planning meeting was held in Niamey in early 1995 and Work Plans and Budgets developed. The Peanut CRSP received $150,000 in the core 1996 budget to support the Work Plan. The funds were supplied under a USAID initiative to allow field Missions to avail themselves of expertise in Global Bureau projects in support of specific country program needs. Dr. Chandra Reddy, Alabama A&M University agronomist with some eight years of project management experience in Niger provided overall leadership for the project. Dr. David Cummins, Program Director, The University of Georgia coordinated areas of infracture support and training of INRAN scientists. This report will describe activities and accomplishments of the project.

MAJOR ACCOMPLISHMENTS

Variety trials in 1995

During 1995, 34 on-farm variety trail were planned and 32 succeeded. Out of this. 25 farmers preferred to grow an improved peanut variety in the future. There were 18 improved varieties tested and only four performed worse than the control variety. These results show that there is opportunity to improve farmer yields with the introduction of improved peanut varieties.

Calcium trials

The application of 300 kg/ha gypsum increased yields of a medium seed size peanut variety by 37% in the Tanda Watershed study for the average of 1995-1996 results . In the same study 300 kg/ha wood ash increased yields of the large seed size variety 40%in 1996 and increased yields in 1995 26% for the small seeded variety and 12% for the medium seeded variety .

At the Konni farm site in 1996, the application of 300 kg/ha gypsum increased peanut yields by 35% and 300 kg/ha rock phosphate increased yields by 30% over the check treatment.

We can conclude that gypsum had produced good response in both years with all seed size varieties, while wood ash was responsive it was more erratic than yield response to gypsum.

Infrastructure

Equipment was supplied for threshing seed, laboratory analysis for aflatoxin, and simple presses for peanut oil extraction.

Training

Training was provided for use of the oil presses and use of the aflatoxin equipment. Consultation for the development of a seed production, multiplication, and distribution system in Niger was supported.

Impediment to progress

In April 1996, just as plans were being made for field activities we were notified not to expend any more funds in Niger due to the overthrow of the elected government. When this restriction was lifted, we were able to salvage part of the program as reported in the accomplishments.

GOALS

The goal of the project is to provide assistance to the USAID/Niger Mission in assuring that technologies developed by the Peanut CRSP and also from other sources (i.e. the Niger National Program , ICRISAT Sahelian Center) are transferred to farmers and processors.

Objectives/Field Activities

An InterCRSP group met with INRAN in early May 1995 for a week-long planning session. The Peanut CRSP component was developed at this time to include the following activities.

1 Farmer field trials. Two levels of on-farm trials are being conducted.

A. Researcher managed trials. These would follow the basic research proposed by ICRISAT/INRAN. They would be limited to two or three trials of each type close to the research stations at Bengou and Maradi.

B. Farmer managed farm trials. These trials will be limited to four treatments, one or more of which can be on one farm. Prime consideration for testing will be given to the newly selected InterCRSP Watershed site at Tanda.

(1). The farmer's variety and practices will be a treatment to compare new interventions against.

(2). Improved varieties. One or two of the eight to ten varieties included in the researcher managed trials will be grown on a farm. All the varieties will be tested using different farmers. Since the trial series would be within a limited geographic area the farmers would be able to evaluate their own test variety and see the other test varieties on neighboring farms.

(3). Date of sowing. Improved yields and reduction in soil insect damage by early sowing will be demonstrated to farmers.

(4). Soil amendments. Wood ashes has proved in research trials to improve peanut yields. Farm trials will be implemented to demonstrate their value. Calcium or lime treatments may be used in some tests as an alternative to wood ashes.

2. Post-harvest activities. Four activities were planned.

A. Village level oil expressors, pickers and shellers will be demonstrated at the farm level. These have the potential to greatly reduce the labor input by women processing peanut.

B. Decontamination of village produced peanut oil. Reports from Senegal show that sunlight can reduce aflatoxin contamination of peanut oil. Tests will be implemented to confirm and demonstrate the process.

C. Training of village women for adding value to peanut. New products such as roasted and sugar coated will be demonstrated. Prior training may be provided by Alabama A&M for the INRAN scientist who will in turn transfer the technology to the village women.

D. ELISA equipment for aflatoxin analyses will be purchased for use in the Crop Quality Laboratory. Simple oil analysis equipment will also be considered for the laboratory.

ORGANIZATION PERSONNEL

Dr. Chandra Reddy, Plant and Soil Science Department, Alabama A&M University coordinator.

Dr. David Cummins, Management Entity, University of Georgia, co-coordinator for infrastructure and training.

Drs. Elena Perez and Victor Nwosu, Food Science Department, Alabama A&M University, resource personnel for post harvest activities.

Dr. Issaka Mahamane, INRAN, country coordinator and coordinator for the soil amendment activities.

Mr. Amadou Mounkaila, INRAN, coordinator for variety trials.

Mr. Moussa Oumarou, INRAN, coordinator for post harvest activities.

RESEARCH ACCOMPLISHMENTS

Variety trials

In 1995, 34 on-farm variety trials were planned and 32 of them were carried out successfully. From these 32 trials, 25 farmers preferred to grow an improved peanut variety in the future. In these trials, 18 different improved peanut varieties were grown and tested on farmer's fields. Only four of them yielded less that the control peanut variety based on pod yields.

The following varieties yielded well. At Mayahi, T177-83 produced 80% more yield than the control, T55-437. At Jiratawa, T169-83 produced 68% and ICGV 87281 produced 403% more yield that the control, T55-437. At Konni, T 131-83 produced 48% more yield than the control, 796. At Dakoro, T14-89 produced 99% more yeild than the control, 796. At Padaria, T169-83 produced 102% more yield that the control, 55-437.

These results show that farm yield can be improved significantly by the introduction and use of improved, adapted varieties.

Agronomic trials

In 1995, a researcher managed trial at Tanda had two varieties: 55-437 (small seeded) and 28-206 (medium seeded). Three levels of gypsum and wood ash were used; control or zero, 150 kg/ha, and 300 for supply of calcium. Wood ash and gypsum gave better yields than the control. With the small seeded variety, the wood ash averaged over rates improved yields by 26%, whereas, with the medium sized seed variety the improvement was 12%. Gypsum averaged over rates increased yields of the small sized seed variety 38% and the medium sized seed variety 44%.

During the rainy season of 1996, two types of agronomic trials were conducted. One test was conducted on the Tanda Watershed south of Niamey. Tanda was selected and developed as a village/watershed site for InterCRSP activities. The other test was conducted on a farm in the village, Konni. Results of these trials follow.

The general objective of the Tanda test was to evaluate the response of different sources of calcium to the yield increase of peanut. Previous work at ICRISAT Sahelian Center showed that wood ash was a good calcium source for peanut. Wood ash and gypsum (a common calcium source for peanut) were compared. Both were applied at 300 kg/ha with a check treatment of no calcium applied. A factorial experiment was used with the three calcium treatments and three peanut varieties; 55437 (small seeded), 28206 (medium seeded), and IC6 87017 (large seeded). The test was repeated on three farms, managed by the researcher. The wood ash and gypsum was applied soon after seed germination and emergence. The test were established timely with the rainy season. Long drought lasting over five weeks occurred in the area, therefore results from only one farm will be reported.

The difference between varieties was small, but the medium seed size variety yielded some higher than the other two.
The response to calcium additions was most apparent. With the medium seed size variety, gypsum produced a 30% yield increase compared to the control (without a supplemental calcium source). Wood ash produced a yield increase only with the large seeded variety; more than 40% increase over the control.

The general objective of the Konni test was to evaluate response to calcium and phosphorus with gypsum (calcium) and Tahoua rock phosphate (phosphate) as sources. The trial was on farm and managed by the researcher. Controls with no amendments were compared with 150 and 300 kg/ha of gypsum and 150 and 300 kg/ha rock phosphate. Yields were increased 35% with 300 kg/ha gypsum and 20% with 300 kg/ha rock phosphate. No response was observed from the 150 kg/ha application of either gypsum or rock phosphate.

It is reasonable to conclude that gypsum produced consistent increases in peanut yields, while response to wood ash was more erratic.

INFRA-STRUCTURE

Aflatoxin detection equipment
Thin-layer chromatography equipment was provided the Crop Quality Laboratory. The equipment will be used to measure aflatoxin levels in peanut grown at the different sites. Recommendations to minimize aflatoxin, a health hazard to animals and humans consuming contaminated products, should be developed.

Oil extraction equipment
Ram-oil presses developed by Appropriate Technology International, Inc. were purchased for INRAN. These were not delivered in time for any results of their use to be presented. These are hand operated, relatively inexpensive, can be produced locally, and are labor and fuel saving. The traditional village extraction process is to boil peanut in hot water for several hours. The seed must be hand crushed with constant stirring in the boiling water. Much wood is consumed during the process in an environment where deforestation and desertification is of major concern.

Threshers
Two threshers were provided primarily for the processing of sorghum at the request to INRAN, which supported the companion Sorghum-Millet CRSP activities.

TRAVEL

To Niger

Drs. Chandra Reddy and David Cummins to Niger for May 1995 planning session.

Dr. Chandra Reddy to Niger in July, 1995 to observe and counsel on research activities.

Mr. Moussa Omarou to Alabama A&M and Vermont for aflatoxin analysis training and oil press evaluation in May, 1996.

TRAINING

Oil Extraction

Mr. Moussa Omarou traveled to the U.S. in early 1996. Drs. Reddy, Nwosu, and Cummins accompanied him to the Better World Workshop in Vermont (the producer of the Ram-Oil Press). Mr. Carl Bielenburg provided training on the use of the press with peanut. This was the first time the press had been used on peanut. It proved very satisfactory. The peanuts were heated whole for 8-10 minutes at 160 degrees F. The whole seed were pressed and about 70% of the oil was extracted.

Mr. Omarou also spent time in the food science laboratory at Alabama A&M to observe aflatoxin analysis and food processing techniques.

Seed production

The Peanut CRSP shared the costs of a consultant, Dr. Lee House, to travel to Niger. He participated in workshops and developed recommendations on the development of a seed production, multiplication, and distribution system. Niger is similar to most developing countries in that it is very difficult for farmers to obtain improved varieties of different crop species.

FUTURE PLANS

Funds for this activity were provided only one year. Subsequently, USAID/Washington through the Africa Bureau has provided a grant to Virginia Tech through the IPM CRSP for InterCRSP work in West Africa. One of the recently approved projects led by Dr. Andrew Manu, Alabama A&M University will have core activities on the Tanda Watershed in Niger. It is anticipated the some of the work started in this project, i.e. gypsum, wood ash and rock phosphate studies, oil extraction demonstrations, introduction of improved peanut varieties, and aflatoxin detection will continue in this follow-on InterCRSP program.

Participating Institutions

United States

Alabama A&M University
The University of Georgia
North Carolina State University
Texas A&M University

West Africa

Senegalese Institute for Agricultural Research (ISRA), Senegal
Senegalese Institute of Food Technology (ITA), Senegal
Institute for Rural Development (IDR), University of Ouagadougou, Burkina Faso
National Institute for Agricultural Research of Niger (INRAN), Niger
Institute of Agricultural Research, Amadou Bello University, Nigeria
Institute of Economic Research (IER), Mali
Food Research Institute, (FRI), Ghana
University of Science and Technology (UST), Ghana

Southeast Asia

Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), Los Baños, Philippines
Institute of Plant Breeding, National Crop Protection Center, Institute of Biotechnology, the University of the Philippines, Los Baños, and the Visayas State College of Agriculture, Philippines
Thailand Department of Agriculture, Kasetsart University and Khon Kaen University, Thailand

Caribbean

Caribbean Agricultural Research and Development Institute (CARDI) Headquarters on the University of the West Indies campus., St. Augustine, Trinidad with primary sites in Jamaica and Belize

Near East

Agricultural Research Center, Giza, Egypt

International Centers

International Crops Research Institute for the Semi-Arid Tropics(ICRISAT), India
ICRISAT Sahelian Center (ISC), Niger
International Development Research Centre (IDRC), Canada
Australian Centre for International Agricultural Research (ACAIR), Australia
Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), France
Conference des Responsables Africains et Francais de la Recherche Agronomique (CORAF), France

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